AN ABSTRACT OF THE THESIS OF

Gerald Walter Beckendorf (Name of Student) for the M.S. (Degree)

in Entomology (Major) presented on April 27, 1967 (Date)

Title: A Comparative Study of the Isozymic Proteins and the Effect of Metachemogenesis in the Tissues of Periplaneta americana (L.)

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Abstract approved: W. P. Stephen

An attempt was made to demonstrate isozymic forms of esterase, MDH, LDH and GPDH and the variation in enzyme pattern with increasing age in the thoracic muscle, appendage muscle and hemolymph of the American cockroach, Periplaneta americana. Sampled specimens of both sexes were aged one through seven days, two weeks and one through six months. The proteins in the muscle homogenates and hemolymph aliquots were separated with the aid of polyacrylamide gel "disc" electrophoresis and it was discovered that the protein concentration should be kept below 4 mg/ml to enable proper photopolymerization of the sample gel. Isozyme patterns were localized on the gel by utilizing histochemical staining procedures. Esterase banding was more pronounced if the pH of the incubation mixture was lowered from 7.0 to 5.6.

Muscle esterase patterns exhibited six fairly consistent major bands and a number of inconsistent minor bands. One effect of aging was the increased resolution of the fastest migrating band on the gel in the muscle of specimens older than seven days.

Non-specific banding in the absence of substrate accounted for the majority of the dehydrogenase activity in the three tissues examined. Muscle MDH-specific patterns were characterized by two minor bands and a major band (R_f value approximately .39) consisting of three subbands. Muscle LDH and GPDH-specific patterns each possessed one specific band with R_f values of approximately .5 and .42 respectively. The density of the main GPDH band varied somewhat in muscle patterns with female thoracic muscle displaying little or no main band activity.

Three major and three to six minor esterase bands were demonstrated in hemolymph. One MDH-specific band was found in hemolymph with an R_f value approximating that of the major three band complex in muscle. Although a number of non-specific bands were displayed in hemolymph, the patterns failed to disclose any LDH and GPDH-specific bands in hemolymph.

Each of the patterns were evaluated as possible biochemical systematic indicators by examining the degree of stability with increasing age. Esterase patterns, although displaying stability in main bands, showed variation in appearance of minor bands for all three

tissues. MDH in all three tissues and muscle LDH displayed a great deal of stability, qualifying these tissue enzymes as candidates for further systematic comparisons. The variation in the density of the GPDH band in muscle and the lack of specific LDH and GPDH banding in hemolymph makes these tissue enzymes of questionable value in future comparisons.

A Comparative Study of the Isozymic Proteins and the Effect of Metachemogenesis in the Tissues of Periplaneta americana (L.)

by

Gerald Walter Beckendorf

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1967

APPROVED:

Redacted for Privacy

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Date thesis is presented April 27, 1967

Typed by Carol Bodine for Gerald W. Beckendorf

To Kathy

ACKNOWLEDGEMENTS

The author is indebted to Dr. W. P. Stephen, Professor of Entomology, for his patience and guidance throughout the course of this investigation.

The author is also indebted to Dr. Oliver Johnson, whose initial instruction and advice on techniques were invaluable.

I would like to thank Dr. R. R. Becker, Associate Professor of Chemistry, for his advice during the course of the experimentation and for his reading of the thesis.

Appreciation is expressed to Dr. E. A. Schwinghamer, Associate Professor of Botany, for time allotments to complete final thesis details.

Thanks are extended to Dr. L. C. Terriere, Professor of Entomology, for the use of instruments and equipment in his laboratory.

Sincere appreciation is also expressed to my parents for their financial assistance and encouragement during the course of this study.

I would also like to thank my colleagues and personal friends whose advice and counsel helped me in the completion of this thesis.

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A COMPARATIVE STUDY OF THE ISOZYMIC PROTEINS AND THE EFFECT OF METACHEMOGENESIS IN THE TISSUES OF *PERIPLANETA AMERICANA* (L.)

INTRODUCTION

An increasing interest has recently been generated in the field of comparative biochemistry as evidenced by the quantity of papers and review articles dealing with this subject. The comparative aspects of biochemical investigation cover a broad spectrum and the studies display diversity with respect to the tissue, enzyme, number and type of species and a realm of other possible variables which may be involved.

The comparative biochemistry of insects has largely been a neglected region of research. One contributing factor has probably been the lack of laboratory instruments with the necessary sensitivity for dealing with microgram quantities of tissue. Many of the past reports have dealt with a section of insect toxicology or an enzymatic assay restricted to one particular enzyme in the total insect body. However, the development of advanced electrophoretic techniques has provided an important avenue in the field of protein study in insects.

Since a large portion of the protein and enzyme research has been performed using electrophoretic methods, it is important that the most sensitive technique displaying the highest degree of resolution be employed. The disc electrophoresis technique utilizing polyacrylamide gels was pioneered by Ornstein and Davis (n.d.) and combined the

advantages of high sensitivity, shorter analysis time with the option of modifiable pore size for differential molecular separation.

An important discovery was that a wide diversity of enzymes display multiple molecular forms which were termed "isozymes," and that these isozymic patterns vary not only within a single organism but even within a single tissue (Markert and Møller, 1959). They also noted that tissue specific patterns arose gradually during development, and that the adult isozyme pattern was attained through gains or losses from embryonic tissues. From studies of sedimentation constants, the isozymes were found to display similar molecular weights but to differ in their complement of charged amino acids (Markert and Appella, 1961). The variation in the isozymes was attributed to the variation in gene pattern which is present in each and every living species (Markert and Møller, 1959). Therefore, it was felt that by examining the isozymic pattern of the tissues of various animal species, much information could be gained concerning their developmental and evolutionary status.

There have been numerous studies performed in an attempt to relate biochemistry to established taxonomic conclusions. Most of this work on insects has utilized hemolymph as the tissue source and much of it is summarized in a review by Wyatt (1961). The hemolymph of fourteen cockroach species, analyzed and compared with hemolymph proteins of two other orthopteroid insects, was found to exhibit species

specificity (Stephen, 1961). Because of the difficulty encountered in obtaining hemolymph from bees, Johnson and Stephen (1964) utilized muscle proteins in their electrophoretic studies, and a distinct generic specificity was found among several representatives of the higher Hymenoptera.

The protein and enzyme investigation on insect development has been primarily devoted to the immature stages which show a large amount of biochemical diversity, especially in the holometabolous insects. Telfer and Williams (1953) described changes in concentration of seven hemolymph proteins during development of the silk moth, Hyalophora cecropia (L.), with one of the fractions appearing late in the 5th instar, maintained through the pupal stage and lost in the adult. Early instar larvae of the silk moth, Samia cynthia (Drury), contain a low concentration of hemolymph proteins, while late fifth instar larvae possess several proteins which are reduced during pupation and diapause (Laufer, 1960a). In both sexes, the protein concentration increases during pupal development. After emergence of the male, the hemolymph protein level drops while the female proteins are retained until they are incorporated into the egg. Loughton and West (1965) noted an increase in the number of hemolymph proteins in several species of Lepidoptera during development. Using paper electrophoresis, Chen (1960) found that the concentration of hemolymph

proteins increased during larval development of Drosophila melanogaster (L.) and Culex pipiens (L.). The total number of protein fractions separated on starch gel was seven and four in Drosophila and Culex respectively. Gilbert and Schneiderman (1961) showed that the amount of hemolymph protein was proportional to the increasing size of the larval stage of the insect. In addition, protein concentration declines as the insect passes from pupa to the adult as protein is being used for adult structures. This phenomenon of lowered hemolymph protein was witnessed in the pupal development of the ant, Formica polyctena Foerster, along with an associated reduction in hemolymph volume (Schmidt, 1965). Using paper electrophoresis, two to five protein fractions were present in ant hemolymph, with the prepupal or early pupal stages possessing the greatest number. Nineteen distinct protein fractions (starch gel electrophoresis) appear during development from egg to adult in Drosophila melanogaster with the terminal adult pattern quite different in appearance from that of the egg (Duke and Pantelouris, 1963). Steinhauer and Stephen (1959) showed three protein fractions present on paper electrophoresis in all stages of development of Periplaneta americana (L.), with exception of the single fraction recoverable from the egg. They also found protein concentration to be dependent upon molting, with particularly the middle fraction reaching a peak of concentration around molting and disappearing between molts. The middle fraction was found to disappear

from males three to four weeks after becoming adults and from females ten days after reaching the adult stage. Siakotos (1960b) found an increase in Periplaneta hemolymph protein concentration during the premolt stage, followed by a gradual return to the original level of the nymphal intermolt stage. He postulates this as a result of transformation of less mobile lipoproteins to lipoproteins with a greater mobility.

Developmental variation is not limited to the immature stages of insects. An extensive amount of investigation has been carried out on hemolymph proteins of the adult American cockroach. Clark and Ball (1956) recorded six fractions for this species; Stephen (1956) recorded two fractions and found these two as well as a dissimilar peak for Blatta orientalis (L.). Siakotos (1960a) demonstrated five fractions of hemolymph proteins in Periplaneta americana of which several were shown to be conjugated proteins. Noticeable changes were discovered in the hemolymph protein and amino acid concentrations of the adult house cricket, Acheta domesticus (L.) from the initially high value at emergence (Nowosielski and Patton, 1965). The protein concentration value attains a plateau in 30 day old adults, after which accelerated effects of senescence begin to take place.

Recently, greater attention has been placed on the effects of development upon enzyme characteristics. Upon examination of

enzymatic activity during development of the cellular slime mold, Dictyostelium discoideum Raper, ten distinct esterases were discovered. The maximum esterase staining occurred during the migrating pseudopodium and culmination stages, whereas staining was minimal in mature spores (Soloman, Johnson and Gregg, 1964). Laufer (1960a) in his studies of insect development and sites of protein synthesis in cecropia and cynthia moths, detected multiple bands of malic dehydrogenase (MDH), lactic dehydrogenase (LDH), α-glycerophosphate dehydrogenase (GPDH) and non-specific esterase. He found at least eight different esterase bands, three MDH bands, one LDH band and two GPDH bands in the hemolymph of cecropia prior to the emergence of the adult (Laufer, 1961). These enzymes maintained tissue and stage specificity and the esterases exhibited substrate specificity, while generally displaying more activity during development of the pupa to the adult.

The succinoxidase activity of the pigmented thoracic muscle of the woodroach, Leucophaea maderae (Fabricus) increases significantly after adult emergence, with its activity in both sexes appearing about equal (McShan, Kramer and Schlegel, 1954). Brooks (1957) observed a positive correlation in the degree of pigmentation with succinoxidase activity in Periplaneta adults and also noted that the two associated phenomenon were characterized by a more pronounced increase in male than female during the initial days of the adult stage. Upon adult

housefly emergence, the level of cytochrome oxidase activity increased 50% from 30 minutes to two hour old specimens and from 100 to 200% in three day old specimens (Sacktor, 1950). Studies on the adult honey bee worker, Apis mellifera L., showed that the level of cholinesterase increased about 15% during the first week to ten days of adult life (Rockstein, 1950). In additional studies on eight to ten day old honey bee workers, the acid phosphatase activity was shown to rise 90% above the level at adult emergence, while the alkaline phosphatase level fell 44% during the same period (Rockstein, 1953). Further work by Rockstein and Brandt (1963) revealed a definite pattern of post-emergent biochemical changes occurring in aging houseflies. He noted a drop in the activity of adenosine triphosphatase in the sarcosomes of flight muscle simultaneous with failure of flight, as represented by the loss of wings after about two weeks. These events were preceded by a decline in the activity of the extramitochondrial fraction of GPDH. The important interrelationship of flight muscle and GPDH activity was pointed out by Zebe and McShan (1957). A high level of DPN-linked GPDH and a low level of LDH were present in highly active flight muscle of insects, while highly active leg muscles had a level of LDH equalling or exceeding that of GPDH. These studies indicate that the maturation of a holometabolous insect initiated in the pupal stage are not really complete with adult emergence, and that there is

an obligatory period of postemergence maturation which Rockstein (1956) termed "metachemogenesis." The age and enzyme relationship may be part of a pattern leading toward biochemical senescence (Clark and Rockstein, 1964).

Esterase patterns have been widely employed in studying enzyme patterns in various tissues and whole body extracts in a variety of insects. Salkeld (1965) found that the number of esterases depended upon the age of the insect and obtained as many as nineteen bands in the one day old nymphal stage of the large milkweed bug, Oncopeltus fasciatus (Dallas). Sims (1965) demonstrated six to seven whole body homogenate esterase bands for Drosophila melanogaster and ten esterases for D. virilis Sturtevant. However, Wright (1963) found that only seven of the ten esterases in D. melanogaster were consistent, while six esterases were reported by Beckman and Johnson (1964). Esterase patterns from whole body homogenates of Periplaneta americana were represented by twelve bands on starch gel (Cook and Forgash, 1965). Esterase patterns show a fairly high level of qualitative and quantitative variation, as shown by the disagreement among authors concerning number of bands present in the whole body homogenate of Drosophila melanogaster. Van Asperen (1964) has also shown that different individuals of the same housefly strain exhibit qualitative esterase variation.

The dehydrogenases in insects have not been extensively reported upon, although a great deal of work has been performed on vertebrate dehydrogenases, particularly LDH. Currently, much of the research is devoted to the genetics and the structural composition of the isozymic forms of LDH. Appella and Markert (1961) demonstrated that the LDH molecule could be dissociated into four inactive subunits by addition of an agent which ruptures the secondary hydrogen bonding of proteins. The subunits exist in two classes on the basis of charge, and by arranging the two subunits in all possible combinations in a tetramer, five LDH isozymes result as determined empirically. Markert (1963) was able to dissociate the tetramer into the four inactive subunits by freezing in salt solution and upon thawing, reassociated the subunits into the reactivated tetramer. By the same process, he was able to take equal amounts of the two homogenous monomers, LDH-1 and LDH-5, mix them and after dissociation and reassociation, obtain the five isozymes in the expected ratio of 1:4:6:4:1. Theories explaining the appearance of subbands of the five principle LDH isozymes have been hypothesized by Costello and Kaplan (1963), Fritz and Jacobson (1965), Koen and Shaw (1965) and Goldberg (1966).

Aside from the work performed by Laufer (1960a, 1960b and 1961) on the dehydrogenases of insects, the work has been meagre at best. Upon examination of the flight and jumping muscle of

Locusta migratoria (L.), two forms of MDH were exhibited, one of mitochondrial and the other of extramitochondrial origin, but displaying different qualitative patterns in flight and jumping muscle (Delbruck, Zebe and Bücher, 1959). Of the dehydrogenases studied in various nymphal and adult developmental stages of Leucophaea maderae, the following complement of isozymes were demonstrated: two forms of MDH in all tissues, one GPDH in muscle extracts and four of the five LDH isozymes in the adult ovaries (Gilbert and Goldberg, 1966).

There has been evidence presented for developmental enzymatic variability in both pre- and post-imaginal stages. The high degree of metachemogenesis makes post-emergent adult development more of a factor than previously expected. If insect zymograms are to be used in an effort to understand more clearly phylogenetic relationships in insects, then the degree of variability among adult populations must be elucidated. Stephen and Steinhauer (1959) utilized amino acids in three species of Periplaneta to determine whether the degree of variation warranted the use of these compounds in taxonomic studies. This thesis study will utilize the same application, but will involve an investigation of esterase, MDH, LDH and GPDH patterns in the thoracic muscle, appendage muscle and hemolymph of male and female adult Periplaneta americana and the variation, if any, with respect to postemergent maturation or metachemogenesis.

METHODS AND MATERIALS

Selection and Isolation of Specimens

Specimens of Periplaneta americana were obtained from a large culture that is currently being maintained at Oregon State University. A large number of late instar nymphs were obtained from the main colony, provided with a standard diet of dog biscuit and water and placed in a large chromatography jar so that molts to the adult could be observed and these specimens isolated. The isolated adults were placed in medium-sized plastic freezer containers approximately seven inches in diameter, again provided with dog biscuit and frequent changes of thinly sliced potato. The date of emergence was then recorded.

When it was possible, both female and male newly emerged specimens were isolated so that they could be sampled simultaneously. Ages of the specimens from which samples were taken were as follows: daily until one week, two weeks and monthly until six months. Unhealthy or dismembered specimens were discarded and not used for analyses.

Dissection and Extraction Procedures

Adults of predetermined age were held at $5^{o}C$ in a refrigerator for approximately thirty minutes prior to hemolymph and tissue

sampling. Cooling served the dual purpose of permitting the separation of individuals desired for study and anesthetizing specimens from which hemolymph was to be taken. These short exposures to cold had no undesirable consequences and non-sampled cockroaches regained their original mobility in a few minutes at room temperature.

The wings of the anesthetized cockroach were removed to better facilitate hemolymph sampling. A jewelers forceps was used to make a dorsal heart puncture between two of the abdominal tergites and hemolymph was withdrawn with a 1.0 ml syringe fitted with calibrated polyethylene tubing having an internal diameter of 0.086 cm and a length of approximately 10 cm. At least 10 μl of hemolymph was withdrawn from each specimen with the sample size averaging about 25 μl. The syringes containing the hemolymph samples were retained at 5°C and out of the light for no longer than 24 hours.

The hemolymph sampled cockroaches were then placed in containers and frozen. Freezing made dissection of thoracic and appendage muscle considerably easier. After an hour in the freezer, the specimen was removed, the appendages were clipped off and a dorsal longitudinal incision was performed on the thorax. Muscle was extruded from the appendages gently, but firmly, by squeezing them with a forceps. The thorax was spread open and the thoracic muscle in its entirety was removed. Care was taken to keep the tissues as cold as possible during muscle removal. These muscle

samples were each placed in previously weighed, especially designed, glass tissue homogenizers containing 0.5 ml of ice-cold distilled water. Generally, the average tissue weight was approximately 80 mg with a variation between samples of 50 to 150 mg. The thoracic and appendage muscle tissues were weighed, the grinders placed directly in the centrifuge and spun for ten minutes at 9000XG to free the tissue of adhering hemolymph and fat body. This step also served to condense the tissue for the grinding procedure which was performed with approximately 50 to 75 μl of ice-cold 0.25M sucrose, added slowly while grinding with the all-glass pestle. This mixture was centrifuged again at 9000XG for ten minutes and the resulting supernatent subjected to either protein determination or polyacrylamide gel electrophoresis.

Protein Determination

Protein concentrations of the samples were determined using two different methods. Periodically, problems were experienced in obtaining polymerization of the muscle extract sample gel prior to electrophoresis and it was suspected that this was the result of the high protein concentration in the extract. No problems were experienced with polymerization of hemolymph sample gels.

The Folin-Ciocalteau protein determination was essentially that of Lowry et al, (1951). The supernatent obtained from the previously described procedures was utilized in the determination. Extracts were

taken from midgut and fat body homogenates, in addition to thoracic and appendage muscle samples. The sucrose volume for one determination was 50 μl while 100 μl was utilized for the second experiment. The Folin samples were analyzed with a Beckman model B spectrophotometer equipped with a blue phototube. The Folin-Ciocalteau phenol reagent (2N) was obtained from Fisher Scientific Company. The protein standard was crystallized and lyophilized bovine serum albumin obtained from Sigma Chemical Co.

The second method was a revision of the basic standard biuret reaction modified by Itzhaki and Gill (1964) to detect low protein concentrations with the corresponding sensitivity of the Folin test. This "micro-biuret" method was used to determine protein in the tissue extracts obtained from single individuals hand ground in the glass homogenizer, and also total protein obtained from the pooled tissue of several individuals ground in a Servall omni-mixer. Samples of muscle tissue freshly frozen and tissue frozen for three days were examined to determine the effects of freezing on protein concentration. Again the protein standard was bovine serum albumin. The micro-biuret samples were analyzed using a Beckman model DB spectrophotometer.

The extraction procedure utilizing the omni-mixer was similar to earlier extraction procedures with the following modifications: pooled thoracic and appendage muscle samples were washed with 1.5

ml of ice-cold distilled water and the distilled water wash was retained for analysis. The muscle samples were carefully removed from the centrifuge tubes and placed in the omni-mixer homogenization chamber along with approximately 0.5 ml of ice-cold 0.25M sucrose. The samples were ground for ten minutes at a medium rheostat setting, the resulting homogenate filtered through glass wool and adjusted to a final volume of 10 ml with 0.25M sucrose. The distilled water wash was also adjusted to a volume of 10 ml with the sucrose solution and 2 ml aliquots of each used for analysis.

To examine the combined effects of omni-mixer extraction and saline versus distilled water wash, 28 day-old females were sacrificed and treated as follows: appendage muscle from four individuals was dissected, rinsed with 1.5 ml ice-cold saline and ground in the omni-mixer as previously described. After filtration the resulting supernatant was adjusted to a volume of 25 ml. From the data on protein levels for omni-mixer extraction, it was assumed that the protein concentration of this diluted supernatant was approximately 1.6 mg/ml. To obtain the appropriate amount for electrophoresis, 0.25 ml (containing 0.4 mg of protein) of diluted supernatant was used. The appendage muscle from two other specimens were treated separately. One sample was rinsed in 0.5 ml of ice-cold saline and the other in 0.5 ml of ice-cold distilled water. Then each sample was individually homogenized in the glass chamber as previously

described. The histochemical patterns of these three samples were then compared after electrophoresis in order to distinguish any enzymatic pattern variation between a sample previously washed with saline and one washed in distilled water.

Polyacrylamide Gel Electrophoresis

The following specialized chemicals were utilized in the course of the electrophoretic procedure: acrylamide monomer, N,N'-methylenebisacrylamide (BIS), N,N,N',N'-tetramethylenediamine (TEMED) and riboflavin which were all obtained from Distillation Products Industries, Division of Eastman Kodak Company; 2-amino-2-hydroxymethyl-1,3-propandiol (TRIS), Nitro Blue Tetrazolium, crystalline Grade III, β-diphosphopyridine nucleotide, Grade III, phenozine methosulfate, L-malic acid, monosodium salt, crystalline dihydrate, DL-lactic acid, sodium salt, grade V, DL- α-glycerophosphate, disodium salt, hexahydrate, Grade X, α-naphthyl acetate and Fast Blue RR salt, all of which were obtained from Sigma Chemical Co.

The procedure adopted was the inversion modification (Davis, 1964) of the original "disc" electrophoresis technique, pioneered by Ornstein and Davis (n.d.). A few modifications were introduced on a Canalco instruction sheet and were incorporated into the procedure. The stock and working solutions were formulated according to Davis (1964) and were made up from their constituent ingredients. One of

the modifications involved decreasing the distance from the light source to the electrophoresis tube during photopolymerization of the stacking gel. In addition, an interval of 30 minutes was allowed for the polymerization of separating gel. The sample to stacking gel ratio was also modified. Approximately 50 μl of extract was routinely combined with 400 μl (0.4 ml) of stacking gel and adjusted to a volume of 800 μl with 40% sucrose. This sample gel mixture would constitute a sample to stacking ratio of about one to eight on a volume/volume relationship.

Electrophoresis was conducted at a current of 2.5 milliamperes per gel column and performed at room temperature. Both a Buchler and a Spinco Duostat Constant current power source were utilized during the course of experimentation. The reservoir buffer was chilled prior to the beginning of this step in order that heating within the gel columns might be kept to a minimum. Bromphenol blue tracking dye was allowed to migrate for a distance of approximately 25 mm which normally took about one hour. Because migration is usually not uniform in each gel column, the total distance from the stacking-separating gel interface to the blue tracking dye front was measured and recorded. This value is important in calculating the relative mobility of each band of enzyme activity.

Gels were removed from the glass columns with the aid of a thin, stiff needle according to the method of Davis (1964). In addition

a medicine dropper bulb was employed in order to properly expel the freed gel.

Histochemical Staining Methods

After the gels were removed from their glass columns, they were emersed in the appropriate histochemical stain. Esterase activity was assayed according to Dessauer (n.d.) with 0.2M Tris-maleate buffer (Burstone, 1962, p. 571) adjusted to pH 5.6. The dehydrogenase activity assay was modified from Goldberg (1963) with nitro blue tetrazolium (NBT), (1.42 mg/ml), diphosphopyridine dinucleotide (DPN), (.428 mg/ml) and phenozine methosulfate (PMS), (.2mg/ml) being utilized with 0.2M Tris-maleate buffer which was substituted for 0.1M Tris buffer. The substrates, 0.1M lactic acid, 0.1M α-glycerophosphate and malic acid (4 mg/ml) were added to evenly divided aliquots of the NBT/DPN/PMS buffer solution.

For a dehydrogenase pattern control, the gels were incubated in the NBT/DPN/PMS mixture with the substrate omitted. Bands appearing on the gel incubated in the presence of each substrate, which did not appear in the mixture without substrate, were judged to be specific to that particular dehydrogenase.

Incubation intervals were varied with each particular enzyme in order to obtain a well-defined pattern which was simultaneously free of over-development. The optimum developmental time for esterases

with all three tissues was found to be 15 minutes at room temperature. Malic dehydrogenase in both thoracic and appendage muscle developed rapidly in ten minutes while blood MDH required 30 minutes at 35°C. All LDH and GPDH gels were incubated for exactly one hour at 35°C. Upon completion of the enzyme incubation, the gels were removed from the stain solution and placed in a storing solution composed of water, methanol, 7% acetic acid (3:2:3) and retained for processing on a Photovolt electrophoresis recording densitometer. The electropherogram scans were examined for peaks of enzyme activity and each peak measured in terms of relative mobility (R_f) with respect to the bromphenol blue front measurement previously recorded.

RESULTS AND CONCLUSIONS

Protein Determination

Extracted protein level was measured by both the Folin-Ciocalteau method and the micro-biuret method and the standard curves are displayed in Figure 1. The two curves for the Folin test closely resembled one another especially at lower protein levels. The micro-biuret standard curve for bovine serum albumin should be linear in the concentration range of 0.026 mg/ml to 0.53 mg/ml (Itzhaki and Gill, 1964) which was exhibited in the second of the two tests. However, the variation between these two micro-biuret curves is probably insignificant at protein levels below 0.5 mg/ml.

The results of the Folin protein determination are reported in Table 1. Values for the total amount of extracted protein in male specimens varied between approximately 0.1 mg and 1.0 mg. Values were given for total protein extracted rather than in protein concentration because variable endogenous tissue water plus the measured sucrose resulted in slightly differential supernatant levels.

The results of the micro-biuret protein determination approximated those obtained in the Folin test and are shown in Table 2. The values for male and female muscle in the first test varied from 0.25 mg to 0.42 mg with the male specimens having a slightly higher

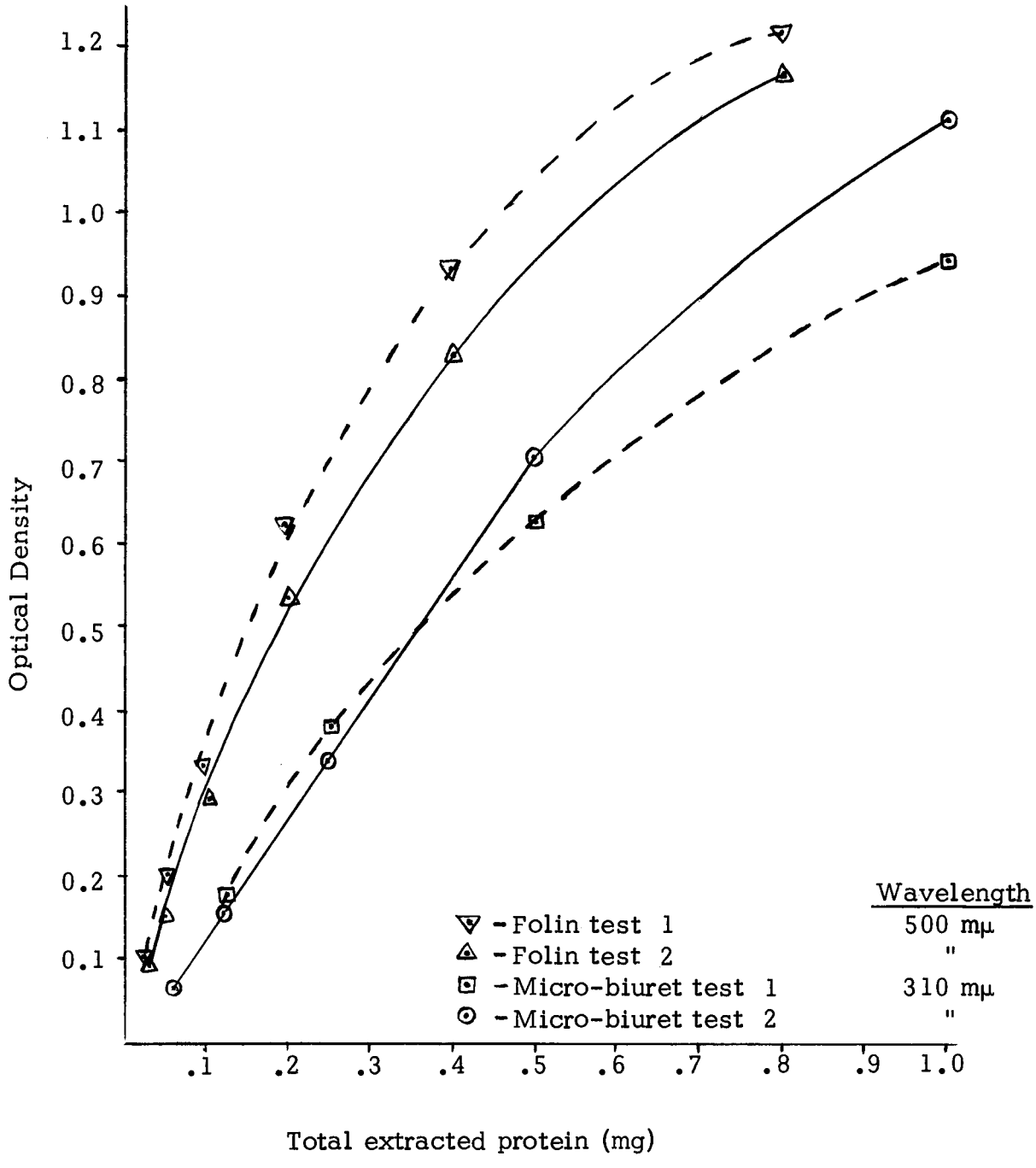


Figure 1. Standard curves for Folin-Ciocalteau and micro-biuret protein determinations.

TABLE 1. FOLIN-CIOCALTEAU PROTEIN DETERMINATION

Test 1

Tissue	Wet weight tissue (mg)	Total extracted protein (mg)
Thoracic muscle	43.9	0.44
Appendage muscle	60.2	0.24
Midgut	4.8	0.09
Fat body	42.1	0.31

Extracted with 50 μl of 0.25M sucrose

Test 2

Tissue	Wet weight tissue (mg)	Total extracted protein (mg)
Thoracic muscle	90.4	0.91
Appendage muscle	71.6	0.57
Midgut	12.3	0.27
Fat body	52.9	high O. D. not determined

Extracted with 100 μl of 0.25M sucrose

TABLE 2. MICRO-BIURET PROTEIN DETERMINATION

Test 1 Glass homogenizer extraction

Tissue	Wet weight tissue (mg)	Total extracted protein (mg)
Female thoracid muscle	101.8	not determined
Female appendage muscle	119.3	0.25
Male thoracid muscle	74.3	0.37
Male appendage muscle	72.3	0.42

Test 2 Glass homogenizer extraction - Variable freezing periods

Tissue	Wet weight tissue (mg)	Total extracted protein (mg)
Female thoracid muscle freshly frozen	46.9	not determined
Female appendage muscle freshly frozen	99.1	0.4
Female thoracid muscle frozen for three days	60.9	not determined
Female appendage muscle frozen for three days	103.7	0.42

Test 3 Omni-mixer extraction

Tissue	Wet weight pooled tissue (mg)	Total protein (mg)	Extract + wash protein (mg)	% protein (% of wet wt.)
Female thoracic muscle wash	770.9	40.1	72.6	9.4
Female thoracic muscle extract	"	32.5		
Female appendage muscle wash	497.0	39.6	74.0	14.9
Female appendage muscle extract	"	34.4		

protein level. Therefore, assuming an average sample volume of about .2 ml, the protein concentration should not exceed 3 or 4 mg/ml for muscle (about 2.5 mg/ml for female thoracic muscle). Higher protein concentrations in the sample gel may discourage photopolymerization. The second test indicated that there was no significant difference in the amount of protein obtained from freshly frozen muscle or muscle frozen for several days. The results of the use of the omni-mixer in protein extraction were shown in the third test. Using the pooled female thoracic and appendage muscle samples, protein levels of 72.6 mg (9.4% protein) from thoracic muscle and 74.0 mg (14.9% protein) from appendage muscle were obtained. The omni-mixer values compare quantitatively with the percentages of protein content in fish muscle (Hamoir, 1955) but the cockroach muscle proteins are extracted at the expense of denaturation of a large portion of the enzymatic activity present in the tissue. This phenomenon was evidenced by the faint staining after electrophoresis of the omni-mixer extract. Comparison of the two samples with distilled water and saline washes showed that, although distilled water does remove approximately one half of the protein, it has little effect on the electrophoretic pattern. The three protein determination tests illustrated in Table II also revealed that protein levels for female thoracic muscle were difficult to determine unless a pooled sample was utilized. The reason for this difficulty is unknown, but it may

have a relationship to the polymerization problem occasionally encountered with female thoracic muscle extracts.

Polyacrylamide Gel Electrophoresis

The polyacrylamide gels which resulted from the electrophoretic survey of the post-emergent development of the American cockroach are reproduced in photographs and diagramatically represented by their densitometric scans. The photographs of the gels and tables of band relative mobility values are arranged according to the developmental sequence of one day to six months for each sex, tissue and enzyme. Therefore, with the study including two sexes, three tissues and four enzymes, the total number of tables and photographs displaying these individual sequences would be twenty four. Unfortunately, space prevents the detailed presentation of all of the electrophoretic scans. However, for each developmental series, examples of scans from both a young and an old specimen are exhibited. In the case of female thoracic muscle, the first eight scans in the sequence are shown in order to display fraction variation.

Non-specific dehydrogenase patterns are represented by a broken line on the scan while the specific dehydrogenase patterns in each case are represented by a continuous line. Photographs displaying an example of specific versus non-specific patterns for each dehydrogenase are also shown (Figures 10, 11 and 40).

Generally, the disc electrophoresis procedure provided excellent results. Occasionally, a problem arose with respect to the failure of the sample gel to polymerize. Gilbert and Goldberg (1966) encountered a similar difficulty with extracts interferring with polymerization. Inhibition occurred more frequently with thoracic muscle than with appendage muscle but could be induced with appendage muscle by the addition of an increased amount of extract. Therefore, it was difficult to determine whether it was the increased protein concentration that was inhibiting polymerization or whether inhibition was due to another factor restricted to the thoracic muscle homogenate. No difficulty was encountered if less than 0.1 ml (protein concentration of approximately 3 mg/ml) of thoracic muscle homogenate supernatent was incorporated with the gel.

Muscle Esterase Patterns

An examination of thoracic and appendage muscle esterase gels revealed a total isozyme complement of sixteen bands. However, there was considerable variation in the total number of bands appearing per gel and in the concentration (peak height) of each of the isozymes on the enzyme scan.

Eight main bands appeared most consistently in female thoracic muscle esterase zymograms (Figure 2) while the total number varied between nine and twelve (Table 3). The recurring bands were numbers

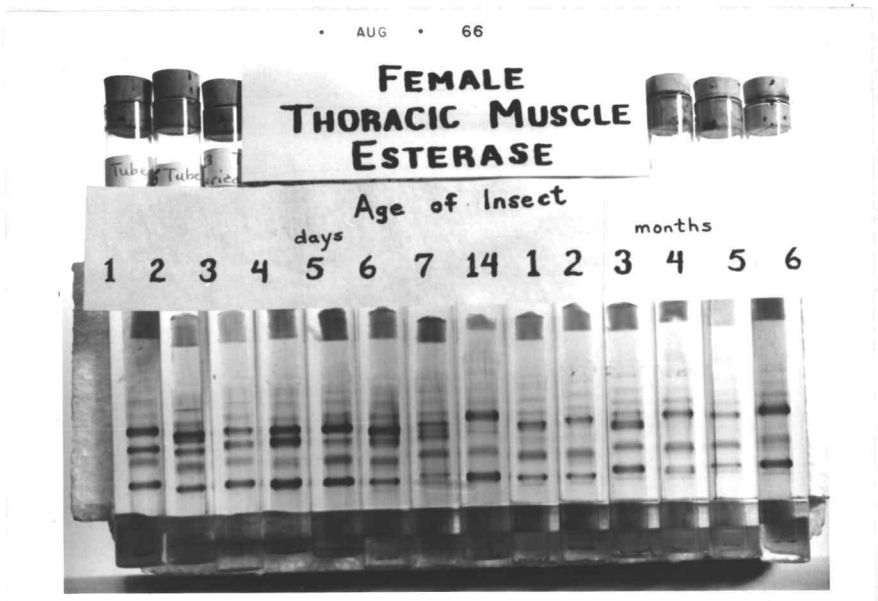


Figure 2. Photograph of female thoracic muscle esterase gels.

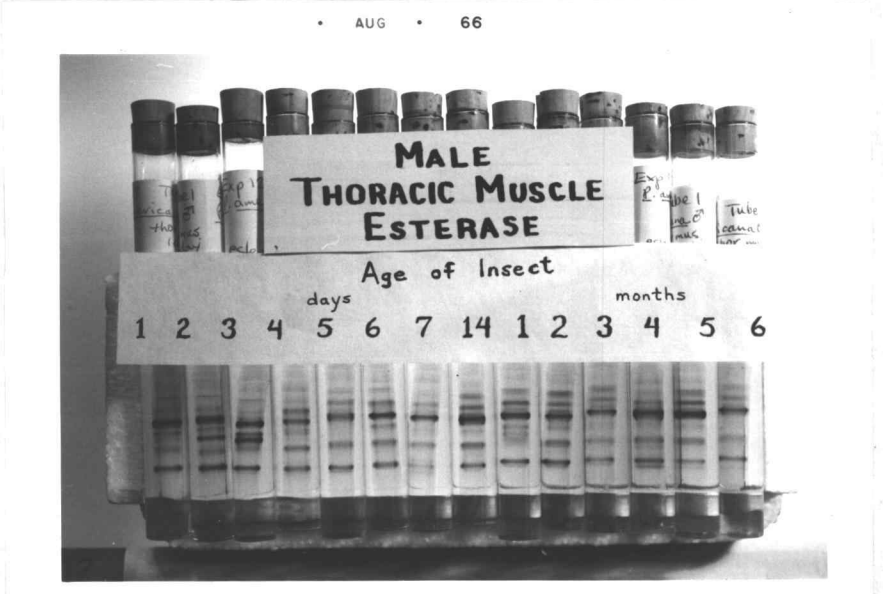
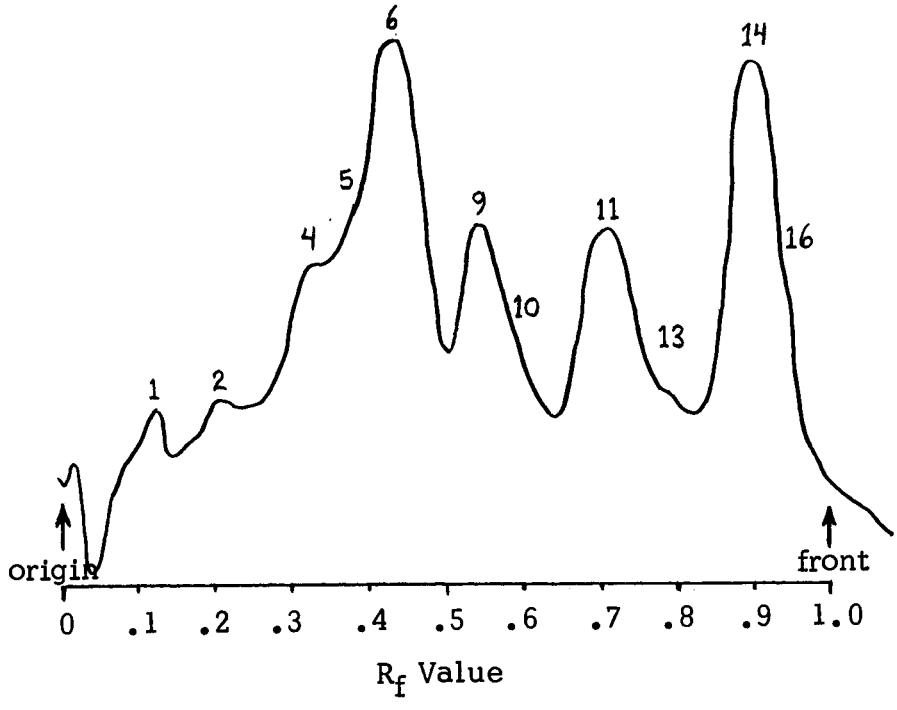


Figure 3. Photograph of male thoracic muscle esterase gels.

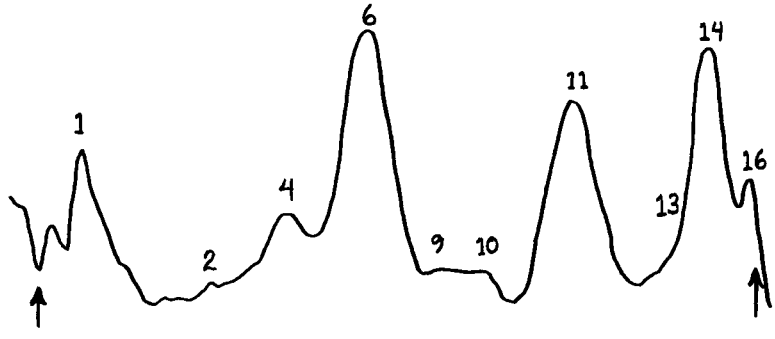
TABLE 3. RELATIVE MOBILITY (R_f) VALUES FOR FEMALE THORACIC MUSCLE ESTERASE

Age	Band Number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total 16 bands
1 day	.095	.178		.303		.428	.447	.511	.602		.712			.91		.95	10
2		.199	.231	.336		.433		.49	.587	.632	.729	.753	.846	.943		.984	12
3		.192	.223	.309	.34	.433			.551	.57	.711		.832	.914		.95	11
4	.12	.194		.337		.409	.445		.511	.535	.714		.821	.903		.938	11
5	.123	.209		.348	.386	.448			.564	.599	.738		.825	.92		.957	10
6	.109	.218		.304		.424	.451		.514	.545	.708	.775		.89		.976	11
7	.113	.221		.321		.413		.486	.538		.715	.79	.86	.92		.987	11
14	.151	.215		.305	.330	.419		.507	.545		.717			.907	.925	.95	11
1 month	.085	.190		.294		.395	.442		.516	.551	.678			.873		.939	10
2	.101	.195		.326		.423			.554		.719	.805		.921		.977	9
3	.096	.187	.202	.302		.397	.428	.492	.512		.698			.885	.9	.98	12
4	.087	.208		.306		.423			.502	.551	.72			.883	.925	.977	10
5	.06	.236		.344		.452			.544	.62	.744		.88	.948		.988	10
6	.093	.189		.304		.426	.452		.585		.696	.767		.889	.907	.982	11

1, 2, 4, 6, 9, 11, 14 and 16, of which bands 2, 4, 6, 9, 11 and 14 predominated (Figure 4a, b, c). Bands number 1 and 16 were weaker and number 9 was subject to considerable quantitative variation. Bands 1 through 6 presented a reasonably stable relationship. On a few gels, band 2 was presented as a double band (band 3) while band 6 occasionally had sub-bands on the trailing edge (5) and the leading edge (7). However, bands 3, 5 and 7 were usually absent. The height of the peak representing band 6 always exceeded that of band 4, both of which exceeded that of 2. Most of the variation encountered was in the region between bands 6 and 14. In specimens up to one week in age, band 9 was strongly dominant and band 10, when present, was very weak, whereas in specimens older than one week bands 9 and 10 decreased quantitatively (Figure 4c). When the R_f value for band 10 was only slightly higher than 9, it then resembled a double band. The R_f value of band 10 was somewhat variable. Band 11, which showed the most consistency, nevertheless did display double band (12) activity several times on its leading edge. A weak band, 13, appeared infrequently while band 14 was always present and prominent. Occasionally, band 14 displayed double band activity (15) and band 16, the fastest migrating fraction, was always present. Usually, band 16 was present on the leading edge of band 15, but in the older specimens there was a greater difference between the R_f value of band 14 and 16 (Figure 4a, b).



a. five day old specimen



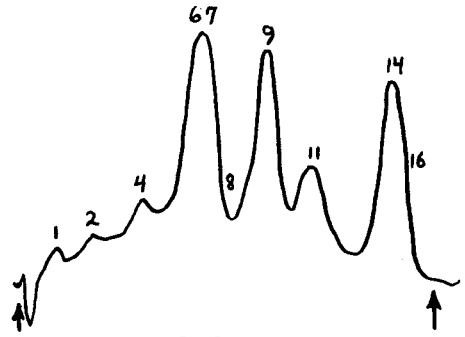
b. five month old specimen

Figure 4. Densitometric scans of female thoracic muscle esterase.

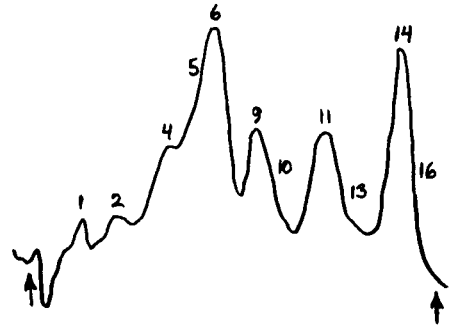
Figure 4. Densitometric scans of female thoracic muscle esterase.

c. one through 14 day old specimens.

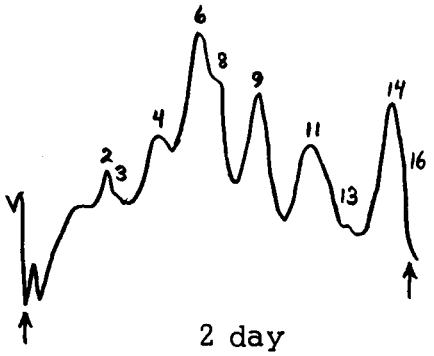
Female Thoracic Muscle Esterase



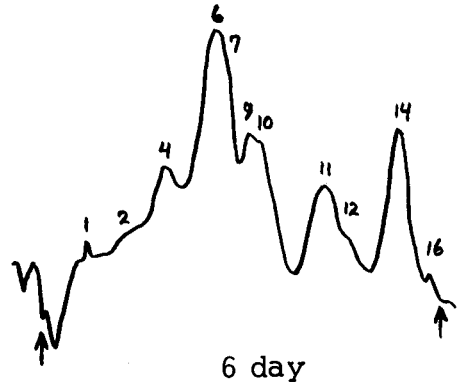
1 day



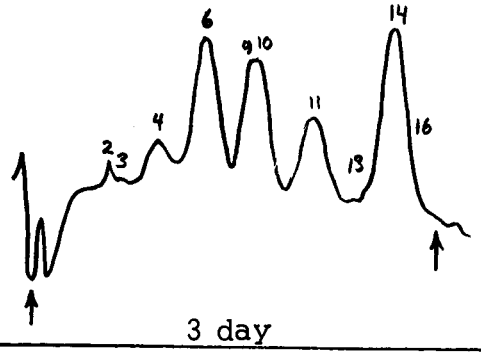
5 day



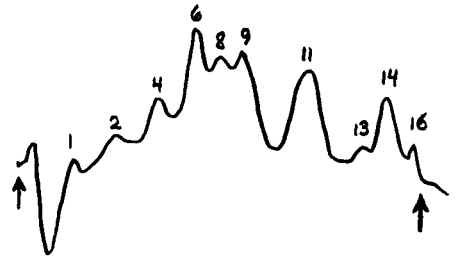
2 day



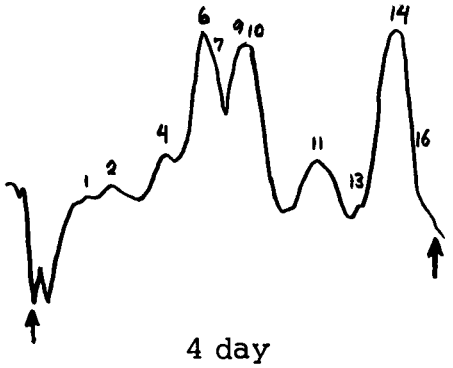
6 day



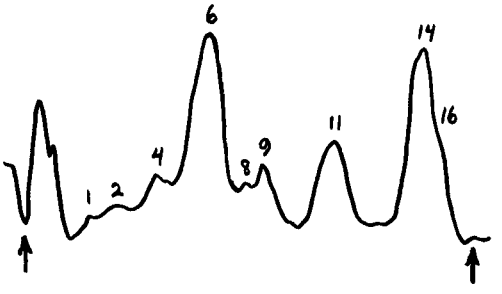
3 day



7 day



4 day



14 day

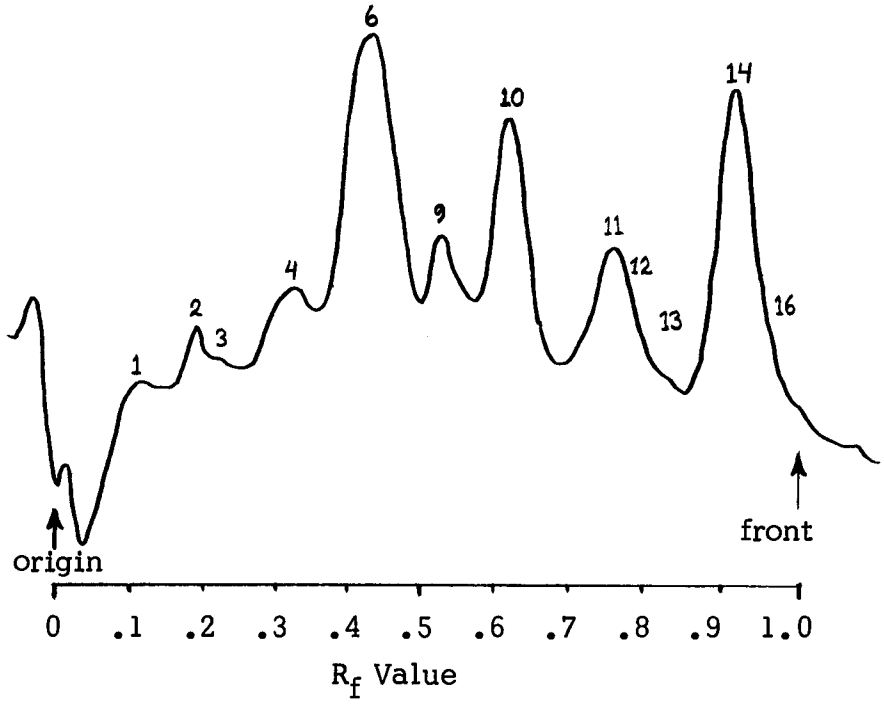
Esterase patterns of male thoracic muscle resembled those of the female with a few exceptions (Figure 3). Band 2 had a greater tendency to show double band activity than in female thoracic muscle (Table 4). Band 5 also appeared more often while band 8 appeared less frequently. However, the greatest variation occurred with respect to band 9 and 10. In male specimens younger than two weeks, the peak height of band 10 (R_f value approximately .61) often equaled or exceeded that of 9. Band 9 peak height in the female almost always exceeded that of either 9 or 10 in the male (Figure 5a, b). This relationship disappeared after two weeks and bands 9 and 10 were weaker in both males and females. Band 13 of male thoracic muscle was stronger than that of the female and the double band at 15 was absent in the male (Table 4). Male patterns have from ten to thirteen bands per gel while the female have only nine to twelve (Table 4).

Esterase patterns of female appendage muscle closely resembled and in some cases were identical to those of the female thoracic muscle. This relationship also seemed to hold in male thoracic and male appendage muscle patterns.

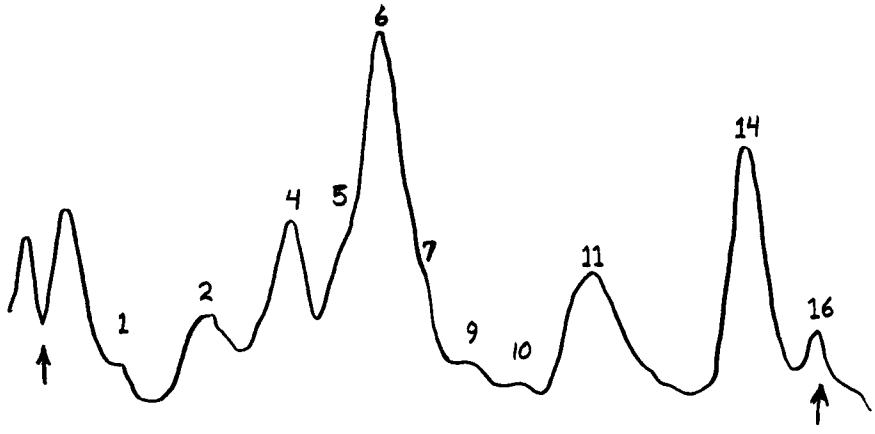
The prominent bands from female appendage muscle gels were the same as those from female thoracic muscle (Figures 6 and 8a, b). Bands 5, 10 and 13 appeared more frequently and bands 8 and 15 less frequently in appendage muscle patterns than in thoracic muscle patterns (Table 5). Band 9 from appendage muscle is not as prominent

TABLE 4. RELATIVE MOBILITY (R_f) VALUES FOR MALE THORACIC MUSCLE ESTERASE

Age	Band Number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total bands
1 day	.106	.184		.318		.439	.478		.549	.615	.705	.729	.804	.914		.96	12
2	.121	.195	.222	.327		.428			.525	.619	.755	.783	.829	.918		.965	12
3	.098	.192		.32		.423	.447		.563	.62	.706			.926		.975	10
4	.09	.176	.211	.317		.426	.458	.528	.555	.614	.715		.848	.926		.973	13
5	.072	.205	.234	.313	.385	.453			.558	.619	.727	.763		.954		.978	12
6	.107	.185	.204	.322		.437			.54	.604	.707		.807	.915		.94	11
7	.094	.185	.21	.322	.427	.445		.518	.572	.619	.714		.87	.915		.978	13
14	.123	.179	.207	.318		.437	.476		.568	.615	.715		.818	.933		.976	12
1 month	.085	.176	.215	.306	.372	.433			.552	.624	.72			.905		.946	11
2	.103	.21	.229	.324	.381	.443	.484		.557		.713		.809	.904		.95	12
3	.095	.206	.24	.312	.368	.432			.552	.594	.722		.801	.905		.954	12
4	.075	.196		.294		.4	.459		.526		.679	.765	.828	.898		.957	11
5	.089	.171	.205	.296	.372	.414			.543		.695		.801	.889		.954	11
6	.1	.215		.322	.385	.433	.493		.548	.623	.707			.870		1.0	11



a. two day old specimen



b. six month old specimen

Figure 5. Densitometric scans of male thoracic muscle esterase.

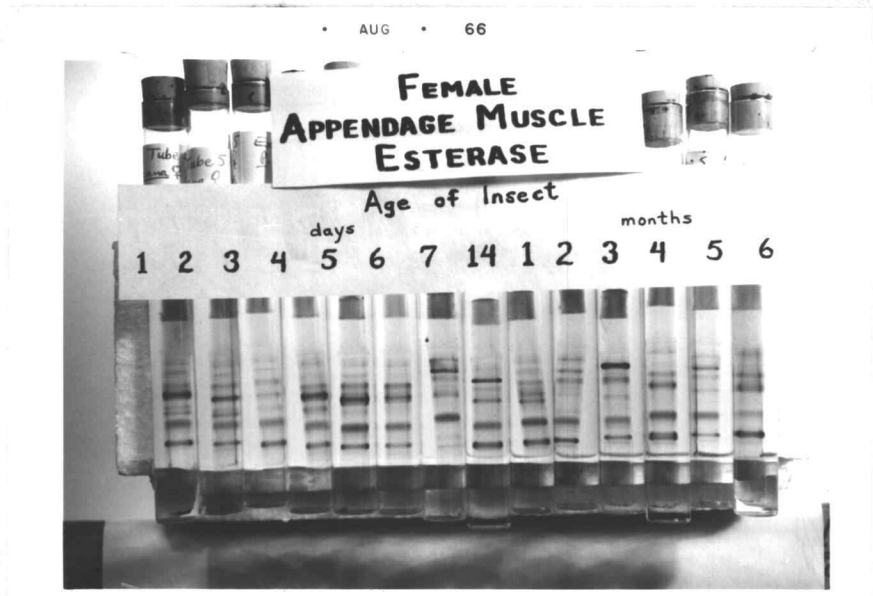


Figure 6. Photograph of female appendage muscle esterase gels.

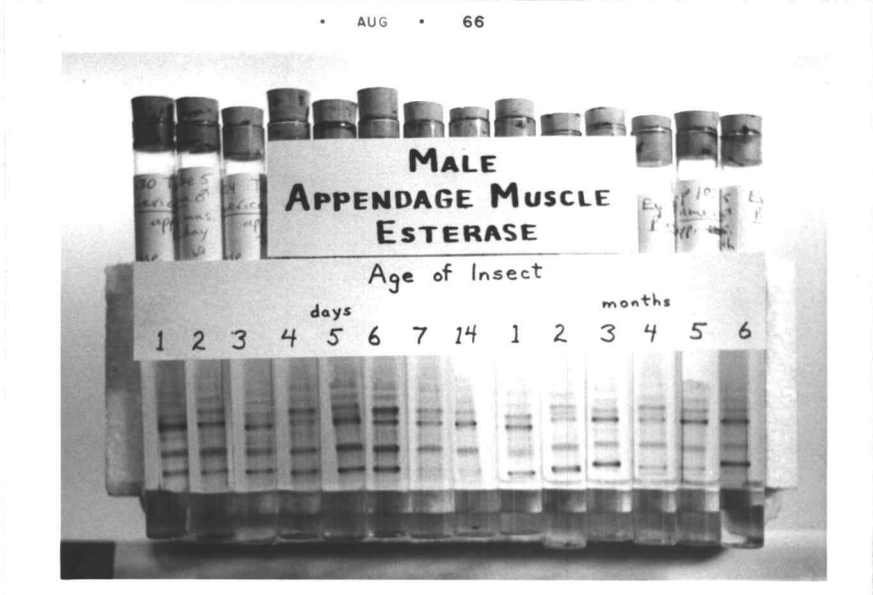
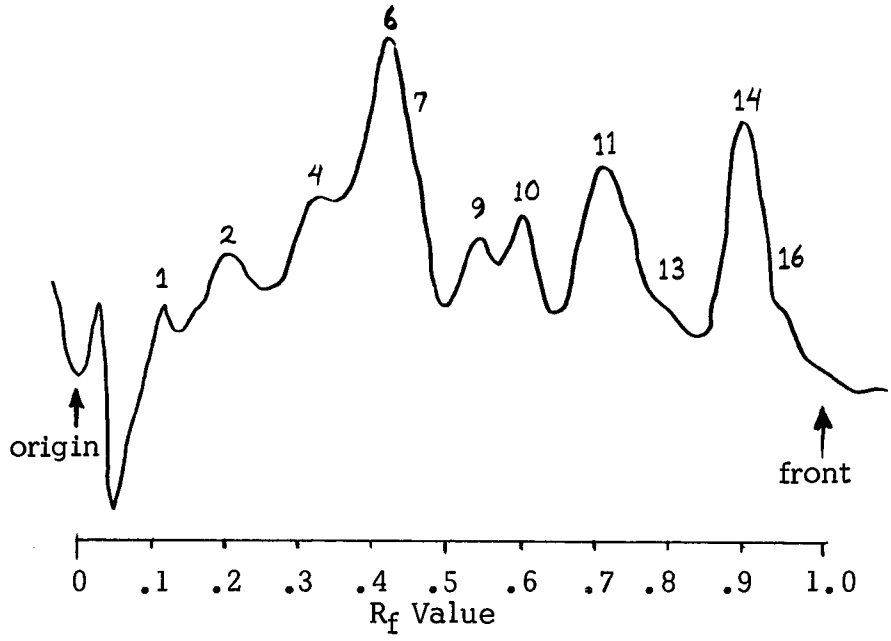
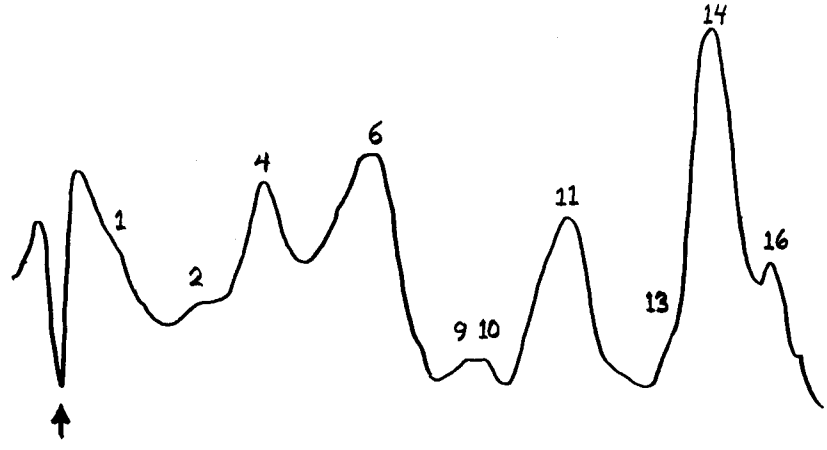


Figure 7. Photograph of male appendage muscle esterase gels.



a. one day old specimen



b. six month old specimen

Figure 8. Densitometric scans of female appendage muscle esterase.

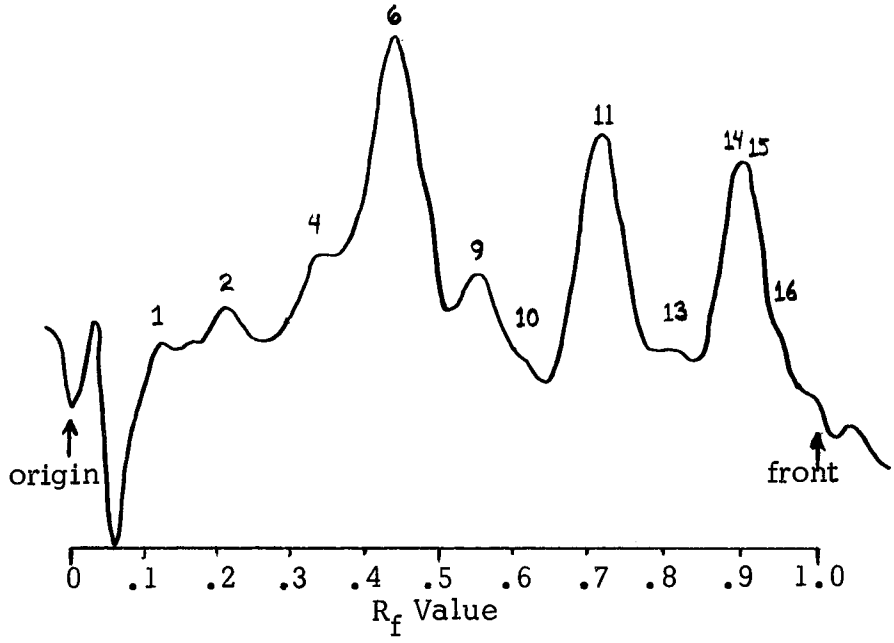
TABLE 5. RELATIVE MOBILITY (R_f) VALUES FOR FEMALE APPENDAGE MUSCLE ESTERASE

Age	Band Number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total 16 bands
1 day	.116	.201		.333		.419	.453		.539	.601	.71		.795	.895		.95 11
2	.106	.188		.306		.431			.549	.6	.69	.71		.883		.933 10
3	.105	.18		.292	.326	.42	.453		.536	.558	.685		.783	.891		.93 12
4	.104	.179	.209	.31		.403	.44		.533	.597	.701		.795	.895		.973 12
5	.087	.192		.298	.385	.434	.472		.562	.581	.728		.827	.918		.975 12
6	.106	.185	.211	.313	.392	.43	.464		.517	.562	.717		.835	.905		.99 13
7	.096	.204		.285	.369	.446			.543		.681			.877		.965 9
14	.084	.219		.316	.380	.447			.557		.726			.937		1.0 9
1 month	.104	.17	.193	.302	.37	.415		.483	.54	.581	.69		.777	.883		.935 13
2	.097	.213		.31	.381	.443			.545	.616	.71		.795	.9		.955 11
3	.121	.212		.275	.388	.45	.471		.55	.596	.721	.77		.912		.97 12
4	.075	.170	.192	.287		.4			.525	.596	.694			.872	.902	.955 11
5	.097	.217		.298	.368	.446			.539	.585	.705		.853	.904		.954 11
6	.082	.196		.282		.431			.549	.573	.667	.698	.824	.895		.972 11

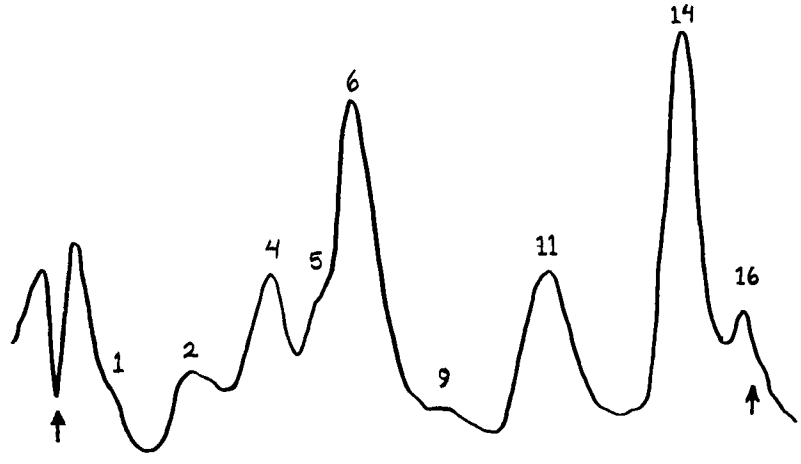
as band 9 from thoracic muscle and often 9 and 10 have a similar concentration in specimens younger than two weeks (Figures 8a, b). In several specimens older than two weeks, bands 2 and/or band 4 may exceed band 6 in peak height but the R_f value for each peak remained quite constant. The total number of bands from female appendage muscle extracts varied between nine and thirteen.

Male appendage muscle patterns also closely resembled those of male thoracic muscle with the exception that in the former band 3 was usually absent (double peak of 2) and peaks 9 and 10 were about equal in magnitude. (Figures 7, 9a, b and Table 6). The total number of male appendage muscle esterase bands varied between eight and thirteen.

All muscle esterase patterns of both sexes showed consistency in six main bands (2, 4, 6, 11, 14 and 16) and the total isozyme complement of each individual varied between eight and thirteen of sixteen possible bands. In a recent study on the esterases of Periplaneta americana, Cook and Forgash (1965), recovered twelve bands from a whole body homogenate but only four bands from muscle tissue. An explanation for the discrepancy in the total number of muscle esterase bands between these two studies may be one of pH of the incubation mixture. Cook and Forgash (1965) utilized a 0.04M phosphate buffer with a pH of 7.15 as compared to a 0.2M Tris-maleate buffer with a pH of 5.6 which was employed in this study.



a. one day old specimen



b. six month old specimen

Figure 9. Densitometric scans of male appendage muscle esterase.

TABLE 6. RELATIVE MOBILITY (R_f) VALUES FOR MALE APPENDAGE MUSCLE ESTERASE

Age	Band Number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total bands
1 day	.128	.206		.342		.43	.471		.55	.611	.716		.806	.883	.903	.953	12
2	.102	.207		.32		.44	.477		.568	.609	.733		.816	.917		.959	11
3	.138	.214		.336	.395	.455			.569	.625	.696	.74	.834	.929		.976	12
4	.092	.176	.205	.304		.407	.447	.502	.543	.586	.69		.829	.899		.95	13
5	.097	.206		.318	.393	.43	.457		.565	.621	.726		.824	.947		.978	12
6	.114	.204		.33	.364	.443			.549	.61	.716		.812	.91		.943	12
7	.131	.242		.335	.4	.454	.504		.589	.631	.735		.89	.94		1.0	12
14		.211		.311	.372	.429	.472		.552	.587	.698		.802	.909		.959	11
1 month	.08	.187		.302		.413	.456		.532	.623	.67			.9		.945	10
2	.092	.206		.321	.386	.439			.534	.630	.725		.828	.958		.99	11
3	.096	.196		.313		.408			.538	.592	.688		.808	.908		.967	10
4		.167		.288	.354	.409	.428		.514	.564	.669		.821	.895		.957	11
5		.188		.305		.414			.559		.688		.789	.886		.953	8
6	.083	.196		.313	.375	.429			.554		.708			.904		.992	9

If the Tris-maleate buffer was formulated with a pH of 7.0, fewer total bands were obtained. When the buffer was adjusted to pH 5.6, the total esterase complement was that which was reported in this study. Although R_f values were not calculated in their study, there was a striking similarity in the number and position of bands from the whole body homogenate and the muscle esterases in the present study. Of the total of twelve esterase fractions in the whole body homogenate, seven of the major fractions may correspond to seven of the major muscle esterases in this study (2, 4, 6, 9, 10, 11 and 14) with band designated E10 and 11 of each study displaying comparable position and heavy density. Of the four muscle esterases, one was densely staining and found in the E10 position mentioned above. The remaining three muscle esterases displayed light staining and were difficult to compare with muscle esterases found in the present study.

Age appeared to have little effect on the <u>total</u> number of muscle esterase isozyme bands (see tables) and quantitative rather than qualitative variation in the fractions was more prevalent. Much of the problem with esterase patterns arises from the fact that as much difference may occur between individuals of the same age as between specimens of different ages, all other factors being equal. Reports dealing with the biochemistry and genetics of houseflies showed that different esterase patterns were obtained from each strain tested and that even individuals of the same strain were highly variable (Van Asperen, 1964). Additional work by Van Asperen and Van Mazijk (1965)

showed that individuals contained three to eight out of a possible ten esterases and that only one of the fractions was present in individuals of all strains. The six constantly appearing esterase bands in Periplaneta therefore represents a relatively stable relationship and these main bands are quite stable throughout the age series from one day to six months. However, the quantitative and occasionally qualitative variation in minor banding in muscle esterase patterns among individuals of the same age, limit the value of this enzyme in phylogenetic comparisons.

Muscle Malic Dehydrogenase Patterns

Muscle dehydrogenase patterns were generally characterized by fewer bands than found for the esterases. Unlike the esterases, they exhibited the interesting phenomenon of banding on the gel in the absence of substrate (Figures 10 and 11). The bands which appeared on the gel incubated in a mixture containing the substrate were judged to be specific for that particular dehydrogenase. Those bands present in the absence of substrate were judged to be non-specific. Upon examination of no-substrate versus substrate gels for the three dehydrogenase patterns, it was determined that the majority of the banding is non-specific in origin. Because a muscle homogenate contains both specific and non-specific dehydrogenase isozymes, possible metochemogenetic effects on each of these types

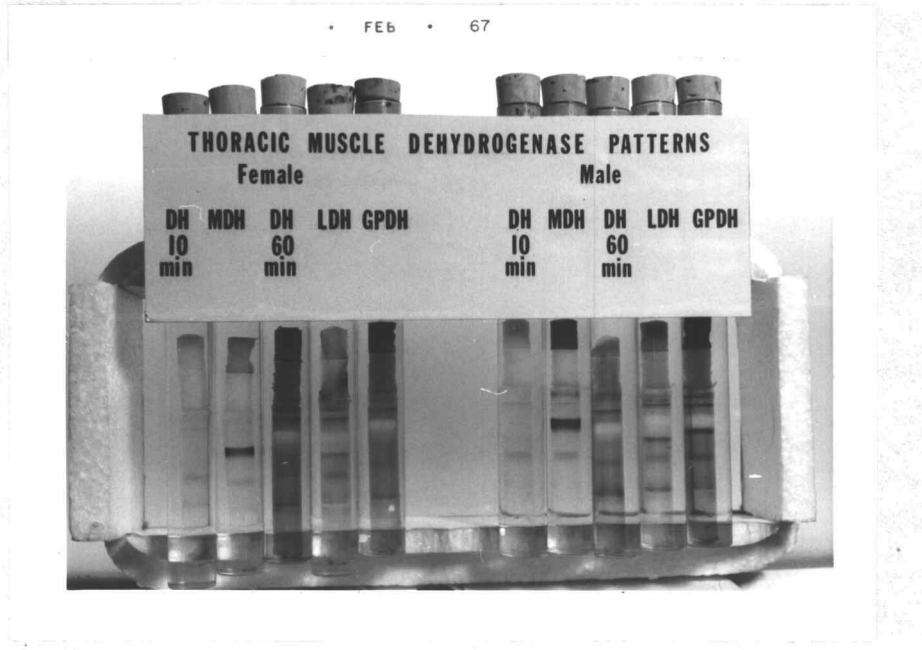


Figure 10. Photograph of specific versus non-specific thoracic muscle dehydrogenase gels.

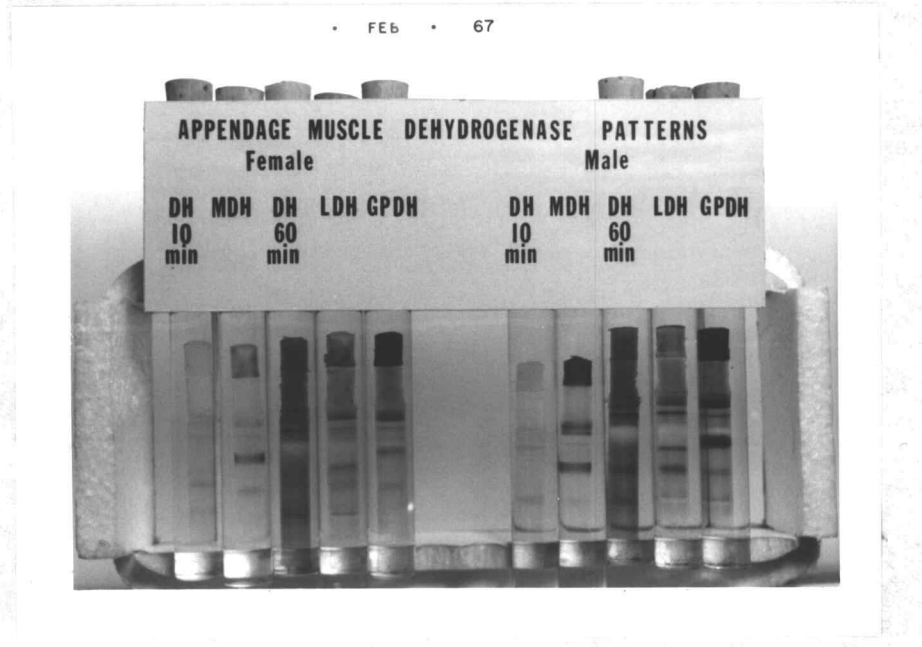


Figure 11. Photograph of specific versus non-specific appendage muscle dehydrogenase gels.

would be important in comparing patterns from different insects.

Thoracic and appendage muscle malic dehydrogenase patterns of male and female displayed an extensively stable relationship with respect to both increasing age and various experiments with specimens of the same age (Figures 12 - 18 and Tables 7 - 10). Therefore, the muscle MDH patterns of both male and female specimens displayed essentially an identical complement of bands. The MDH patterns were simple compared to those of the esterases as there are only three major and two minor MDH-specific bands in muscle. Three of these bands (7, 8 and 9) were usually not resolved as discrete isozymes on the densitometer but rather as one large peak. Note the lightened areas within the main peak and the occasional line representing the middle band in the center (Figures 11, 12, 16 and 17). Only by visual determination can the bands be seen. Of the four minor slow migrating bands on the trailing edge of the major peak complex, two (5 and 6) appeared to be MDH-specific (Figure 14a, b). A major non-specific band (10) did not display the purplish tint resulting from neotetrazolium staining and therefore was not indicative of dehydrogenase staining. This orange band was found in all muscle dehydrogenase patterns and consequently its R_f value was calculated.

Bands 1 and 2 displayed varying peak magnitude depending on the age of the individual. Band 1 was represented by a small peak on the trailing edge of band 2 in specimens up to two weeks in age

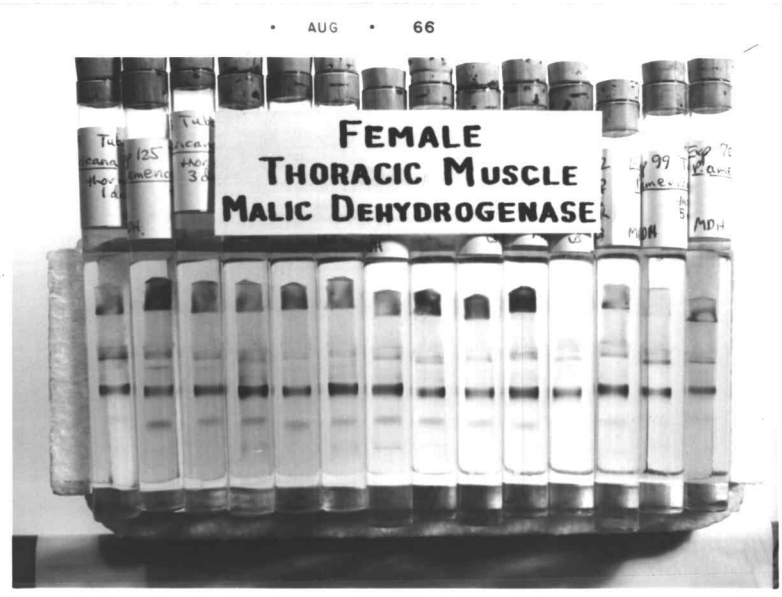


Figure 12. Photograph of female thoracic muscle malic dehydrogenase gels.

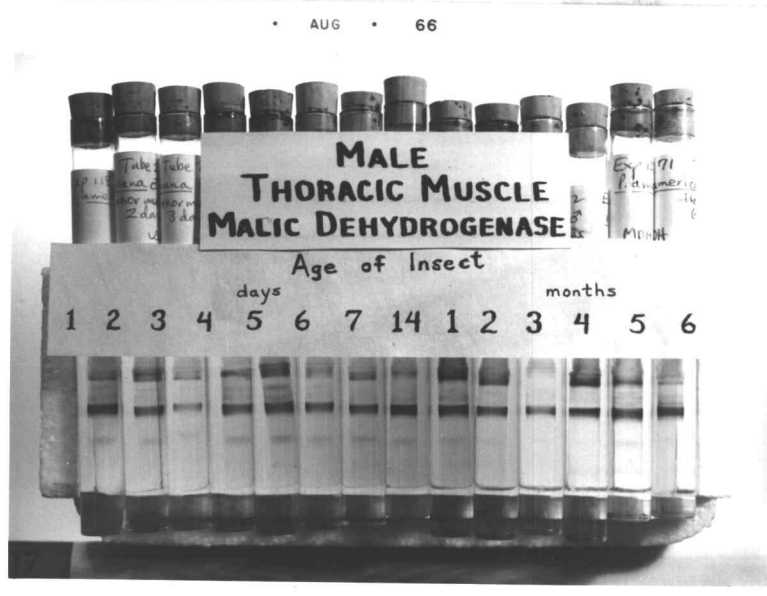
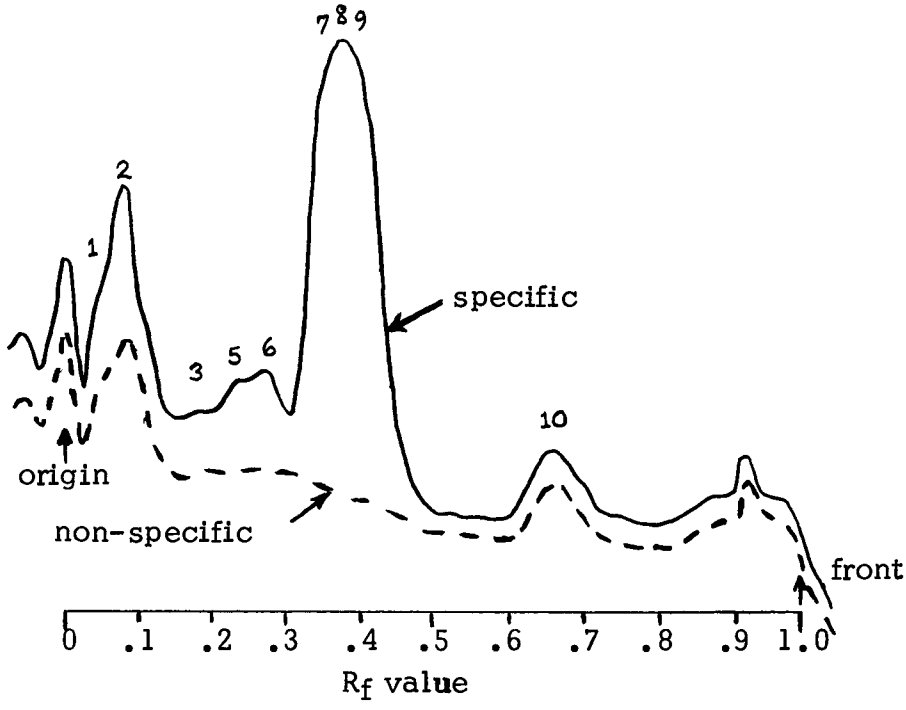
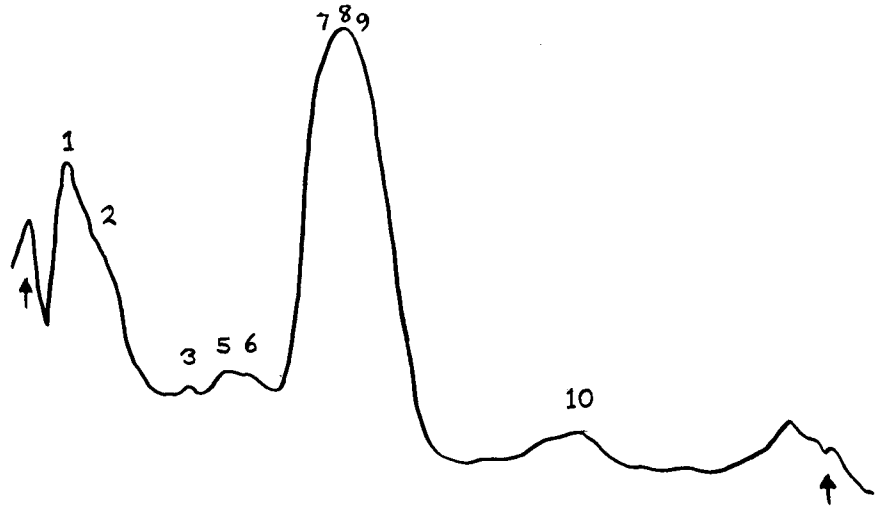


Figure 13. Photograph of male thoracic muscle malic dehydrogenase gels.



a. one day old specimen



b. six month old specimen

Figure 14. Densitometric scans of female thoracic muscle malic dehydrogenase.

Figure 14. Densitometric scans of female thoracic muscle malic dehydrogenase.

c. one through 14 day old specimens.

Female Thoracic Muscle Dehydrogenase

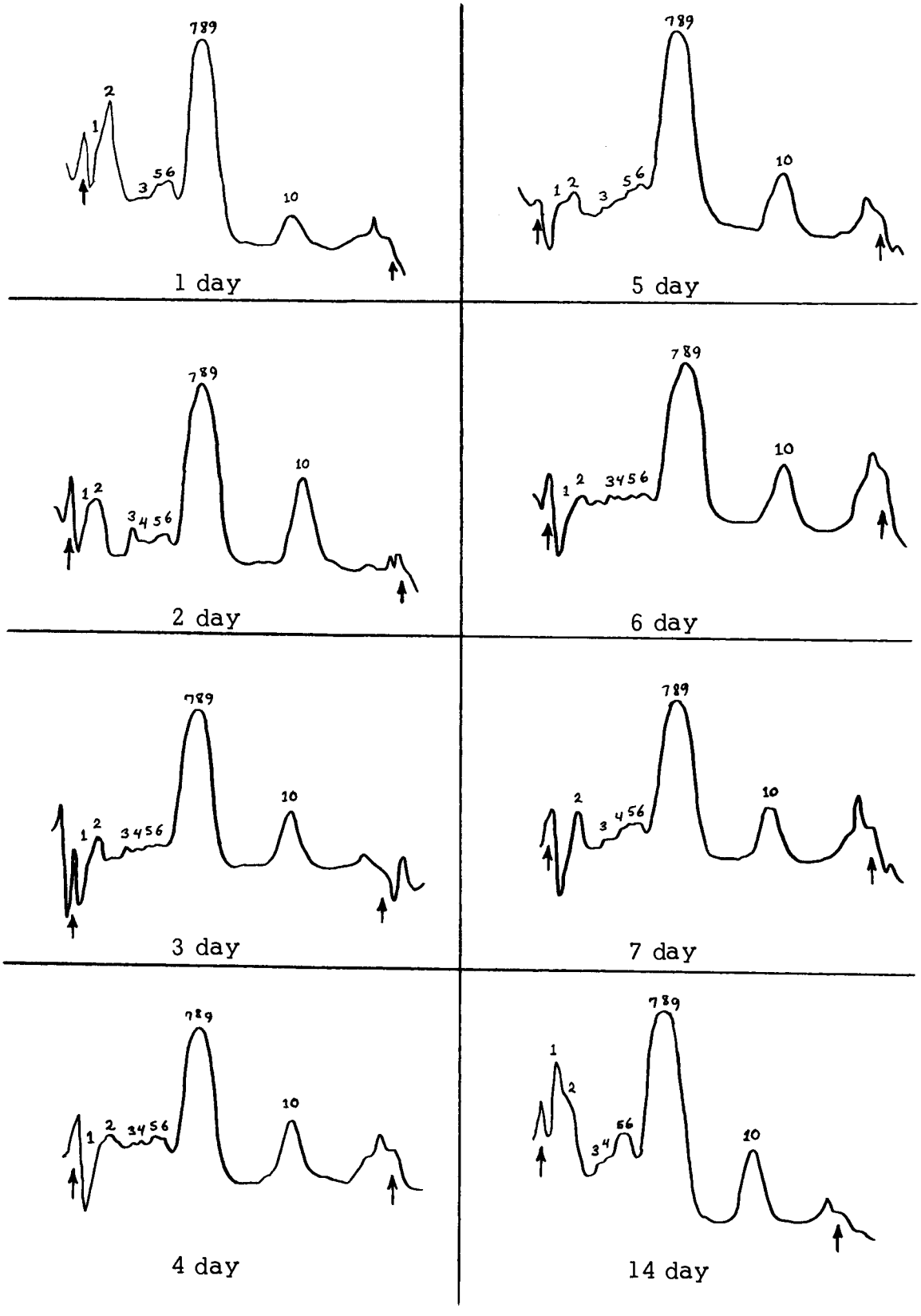
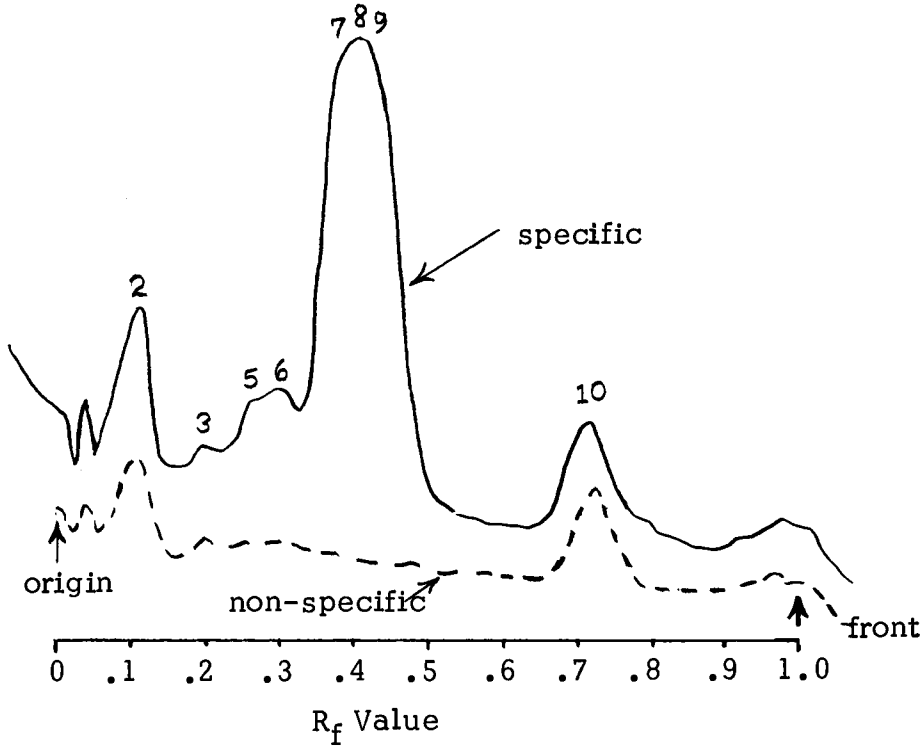
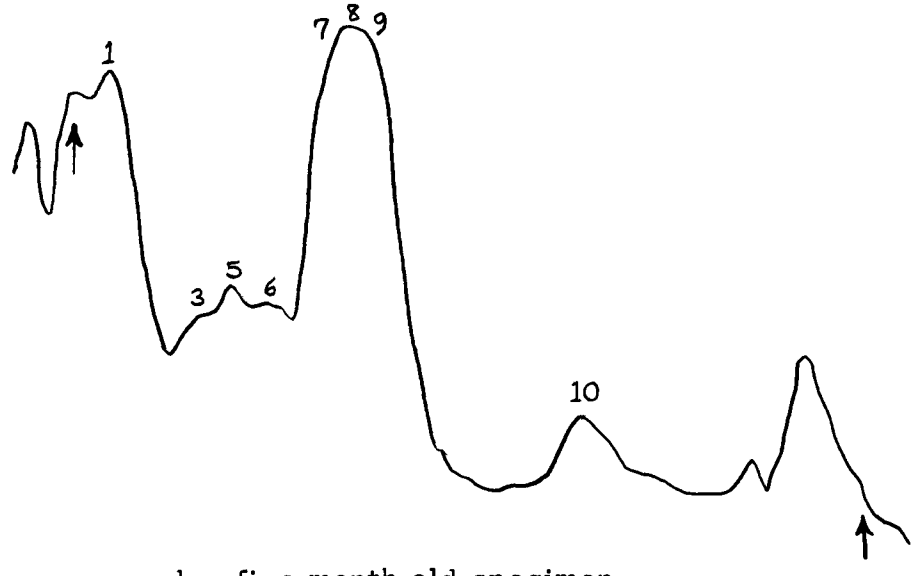


TABLE 7. RELATIVE MOBILITY (R_f) VALUES FOR FEMALE THORACIC MUSCLE MALIC DEHYDROGENASE

	Band Number									
Age	1	2	3	4	5	6	7	8	9	10
1 day	.047	.083	.169		.236	.272	.346	.382	.421	.67
2 day	.048	.078	.185	.218	.258	.295	.362	.395	.428	.697
3 day	.047	.075	.17	.205	.237	.276	.347	.386	.426	.674
4 day	.055	.102	.173	.2	.243	.278	.353	.388	.416	.682
5 day	.064	.107	.192		.263	.302	.358	.391	.429	.711
6 day	.056	.098	.188	.211	.252	.29	.368	.398	.436	.71
7 day		.078	.168	.195	.242	.269	.362	.389	.424	.689
14 day	.058	.103	.194	.223	.26	.298	.376	.417	.455	.719
1 month	.058	.105	.198		.26	.295	.372	.415	.454	.706
2 month	.048	.081	.214		.258	.298	.35	.397	.441	.71
3 month	.042	.081	.169	.223	.281	.312	.404	.446	.484	.708
4 month	.06	.094	.211		.275	.309	.4	.443	.479	.736
5 month	.032	.075	.185		.236	.276	.355	.394	.433	.693
6 month	.027	.081	.188		.238	.297	.373	.419	.458	.712



a. one day old specimen



b. five month old specimen

Figure 15. Densitometric scans of male thoracic muscle malic dehydrogenase.

TABLE 8. RELATIVE MOBILITY (R_f) VALUES FOR MALE THORACIC MUSCLE MALIC DEHYDROGENASE

Age	Band Number 1	2	3	4	5	6	7	8	9	10
1 day		.106	.192		.259	.298	.377	.404	.435	.722
2 day	.023	.079	.189		.249	.287	.362	.4	.434	.698
3 day		.095	.207		.265	.298	.364	.397	.43	.72
4 day	.035	.087	.177	.201	.244	.272	.347	.382	.418	.701
5 day	.04		.186		.237	.276	.348	.387	.43	.715
6 day	.048	.096	.185	.211	.255	.288	.365	.391	.421	.697
7 day		.093	.189	.215	.263	.304	.363	.389	.423	.696
14 day		.065	.165	.203	.245	.283	.36	.387	.418	.686
1 month	.050	.070	.209		.264	.302	.361	.391	.422	.717
2 month		.090	.208		.261	.314	.368	.4	.445	.747
3 month			.154		.216	.262	.366	.396	.446	.707
4 month	.038	.096	.192		.235	.262		.358		.685
5 month	.048		.171		.204	.253	.316	.353	.405	.658
6 month	.052		.17		.256	.296	.33	.382	.43	.7

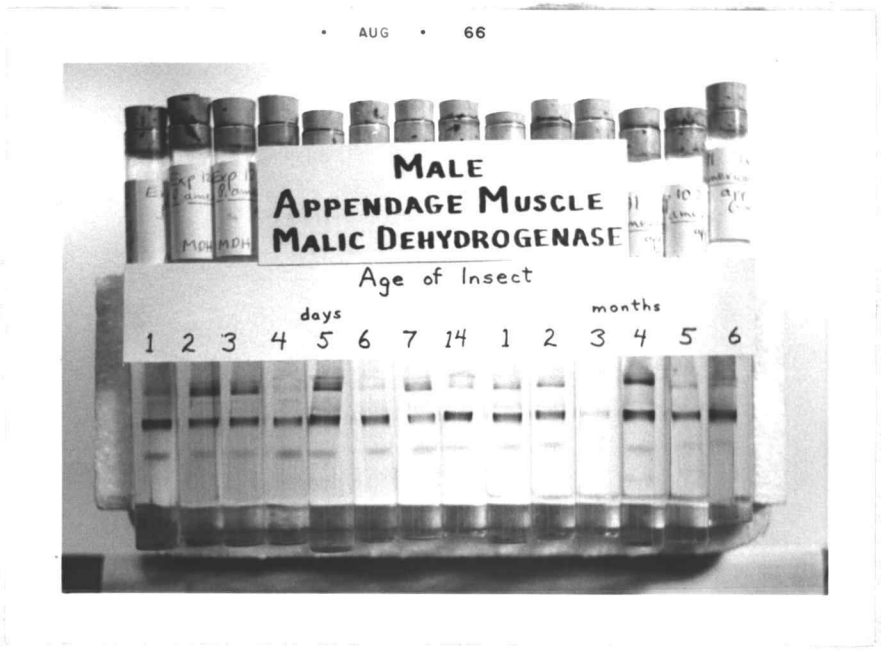


Figure 16. Photograph of female appendage muscle malic dehydrogenase gels.

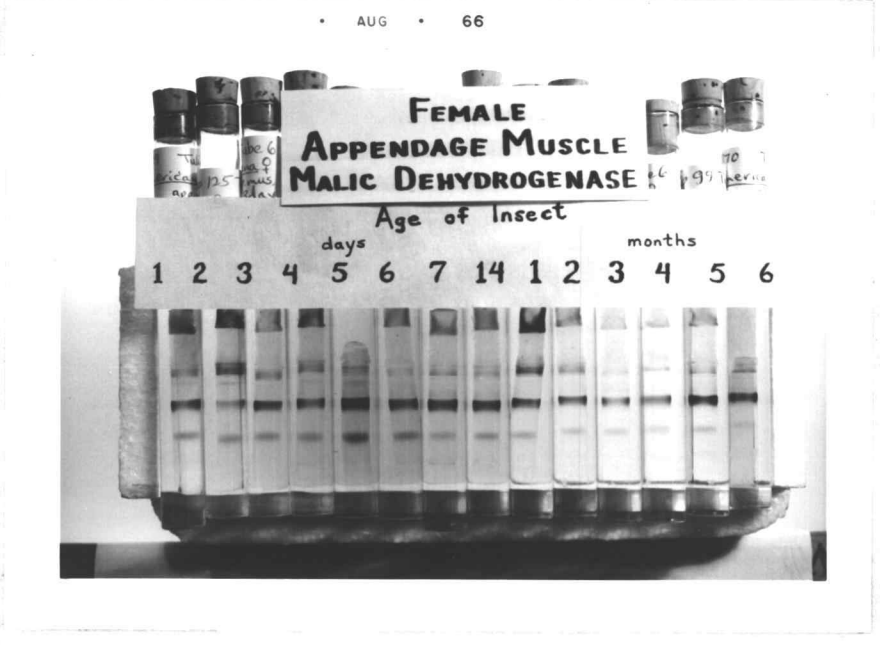
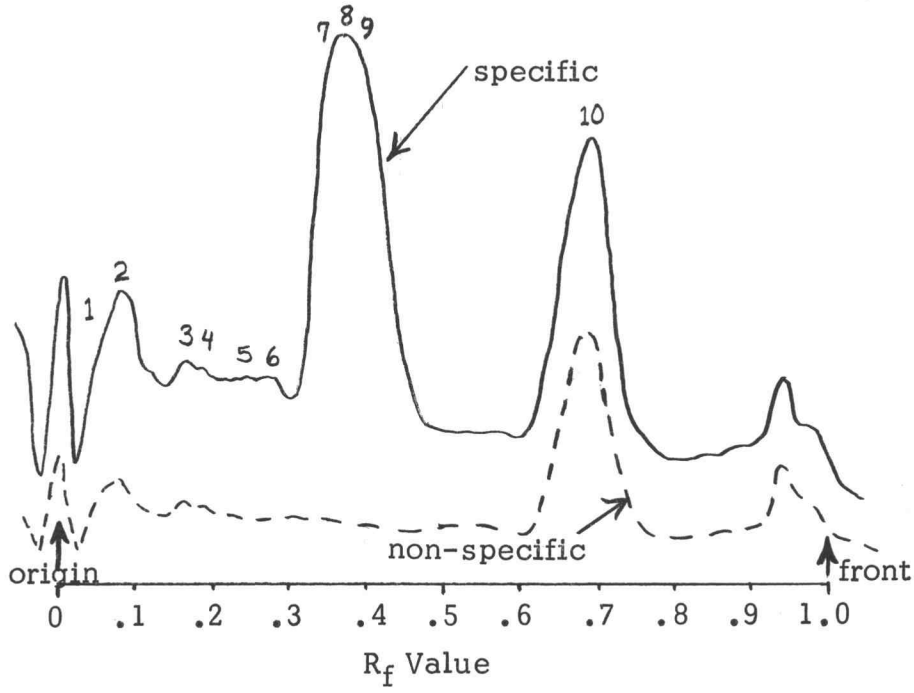
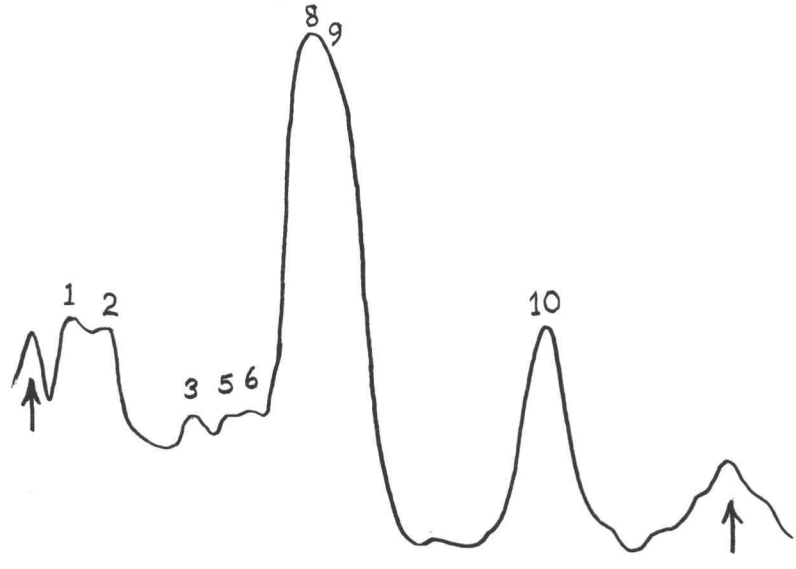


Figure 17. Photograph of male appendage muscle malic dehydrogenase gels.



a. one day old specimen

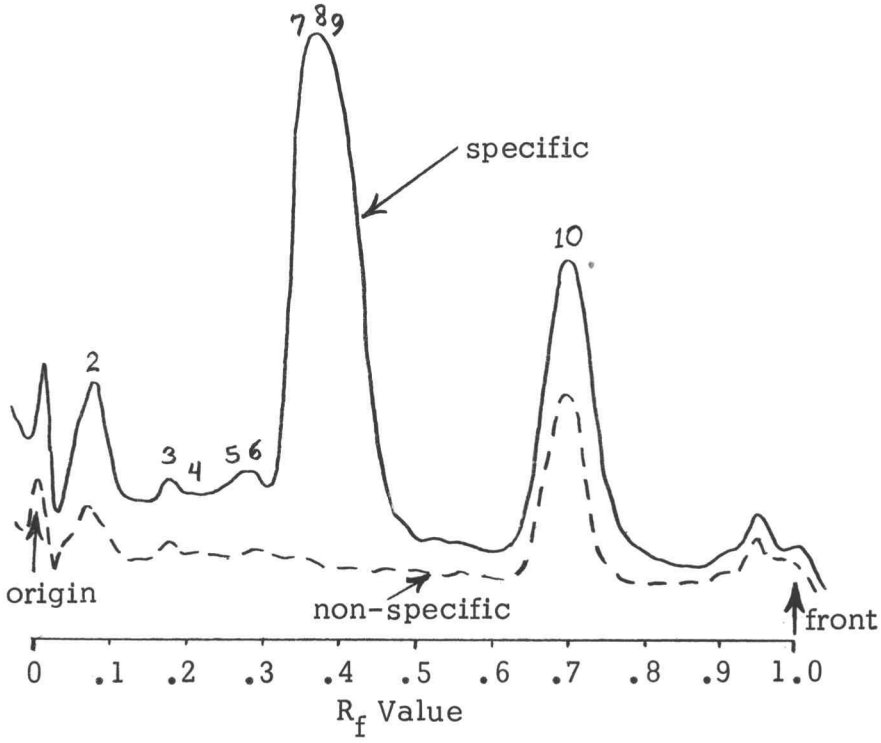


b. five month old specimen

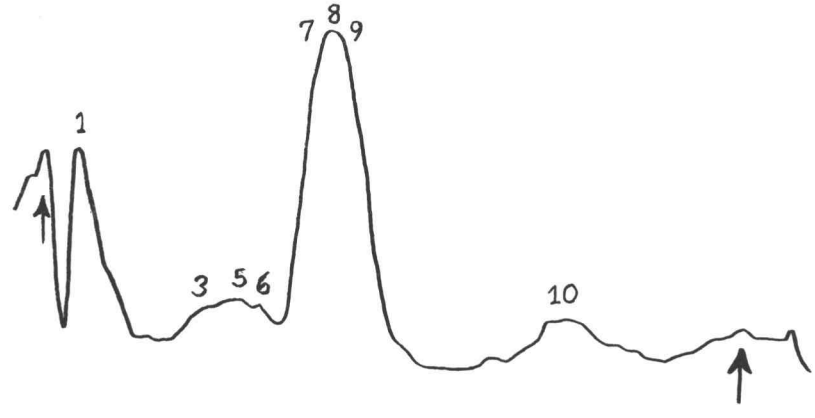
Figure 18. Densitometric scan of female appendage muscle malic dehydrogenase.

TABLE 9. RELATIVE MOBILITY (R_f) VALUES FOR FEMALE APPENDAGE MUSCLE MALIC DEHYDROGENASE

	Band Number									
Age	1	2	3	4	5	6	7	8	9	10
1 day	.045	.076	.159	.181	.234	.276	.343	.37	.408	.69
2 day	.04	.073	.178	.2	.236	.273	.356	.393	.425	.71
3 day	.047	.11	.205	.228	.268	.303	.354	.382	.421	.716
4 day		.068	.168		.239	.279	.35	.389	.425	.704
5 day	.065	.11	.219		.24	.308	.359	.391	.43	.706
6 day		.102	.181	.219	.264	.313	.366	.393	.43	.743
7 day		.083	.169	.187	.247	.278	.345	.375	.405	.694
14 day	.052	.086	.16	.19	.227	.272	.335	.357	.383	.673
1 month	.054	.089	.197			.279		.353		.631
2 month	.044	.075	.214		.258	.294	.349	.381	.413	.71
3 month	.047	.11	.213		.26	.284	.37	.398	.433	.72
4 month	.050	.077	.192		.258	.3	.377	.404	.439	.712
5 month	.061	.107	.229		.279	.320		.398	.447	.734
6 month	.049	.071	.196		.233	.26		.346		.605



a. one day old specimen



b. three month old specimen

Figure 19. Densitometric scans of male appendage muscle malic dehydrogenase.

TABLE 10. RELATIVE MOBILITY (R_f) VALUES FOR MALE APPENDAGE MUSCLE MALIC DEHYDROGENASE

Age	Band Number 1	2	3	4	5	6	7	8	9	10
1 day		.065	.169	.184	.234	.276	.338	.36	.399	.697
2 day	.054	.085	.185	.209	.247	.278	.347	.378	.417	.66
3 day	.042	.085	.173	.208	.246	.281	.323	.358	.392	.685
4 day	.052	.101	.18	.199	.24	.277	.349	.39	.431	.693
5 day	.032	.086	.169	.202	.245	.281	.349	.381	.414	.712
6 day		.074	.171	.201	.264	.297	.35	.376	.42	.695
7 day	.058	.096	.193	.212	.262	.3	.354	.385	.415	.711
14 day	.050	.104		.219	.265	.308	.368	.392	.423	.712
1 month	.053	.101	.219	.2	.271	.296	.364	.397	.429	.73
2 month	.048	.084	.196		.256	.292	.36	.392	.428	.72
3 month	.05	.095	.234		.28	.304	.374	.407	.448	.74
4 month	.051	.081	.206		.261	.283	.357	.393	.430	.724
5 month	.059	.126	.221		.265	.308	.392	.419	.459	.731
6 month	.043		.189		.232	.254	.314	.343	.378	.593

(Figure 14a, b). After two weeks, the peak corresponding to band 1 equaled or exceeded the height of band 2. Bands 3 through 6 were weak and often while they could be determined visually, could not be adequately resolved by the densitometer (Figure 14c). One influential factor in resolving these bands was time of development. Over-development caused bands 3 through 6 to be more prominent but it simultaneously fused bands 7 through 9 making differentiation difficult. Both male thoracic and male appendage showed a greater tendency to display the minor MDH-specific bands 5 and 6. Aging caused bands 3 through 6 to become less prominent and often one or more disappeared. After two weeks, examined specimens had only three bands in the band 3 to 6 region but it was difficult to determine if the slowest migrating fraction was 3 or 4. Although the R_f value was somewhat high for 3, it most closely approximated its position and was included as that fraction arbitrarily. Aging also had an effect on bands 7 through 9 causing the number of resolved bands in this vicinity to be reduced to two or one. However, most of the specimens displayed the total complement of three bands.

The total MDH-specific complement, consisting of a major band complex with three sub-bands and two minor bands, in Periplaneta exceeds that recorded for several other orthopteroid insects. The flight and jumping muscle of Locusta migratoria yielded two forms of MDH, one of mitochondrial and one of extra-mitochondrial origin

(Delbruck, Zebe and Bücher, 1959). Different qualitative patterns in flight and jumping muscle were also noted in Locusta. On polyacrylamide gel MDH also displayed two forms in all tissues of Leucophaea maderae (Gilbert and Goldberg, 1966). The main band of the two Leucophaea MDH isozymes occurred in the vicinity of the main densely staining three sub-bands of Periplaneta found in this study, whereas the smaller Leucophaea band closely corresponds to the moderately staining non-specific band 3 in Periplaneta. Aside from the smaller bands displayed in Periplaneta, there appears to be a close R_f relationship in the MDH isozymes of these three insect species which are indeed phylogenetically related.

As previously stated, the differences found between male and female muscle MDH of the same age were minimal. The qualitative and quantitative variation with increasing age was also quite small. The overwhelming stability displayed by MDH in Periplaneta muscle tissue would qualify this enzyme as a possible systematic indicator.

Muscle Lactic Dehydrogenase Patterns

Studies of lactic dehydrogenase showed them to exhibit non-specific staining in the absence of lactic acid substrate. Upon comparison of the no-substrate pattern with that obtained with the addition of substrate, it was found that the majority of the bands were non-specific (Figures 10 and 11). The non-specific banding was more

intense in these gels as they were incubated for one hour compared to the ten minute incubation time for MDH non-specific patterns.

LDH patterns in Periplaneta muscle were characterized by one specific band (8) and five main non-specific bands including 2, 4, 5, 9 and 10 (Figure 22a). The orange non-specific band 9 resembled band 10 described under the section covering MDH patterns both as to appearance and R_f value. Band 1 also had the same R_f value as band 1 in MDH patterns. As in MDH, band 1 of newly emerged adults was very weak but became more prominent in two week old adults (Figure 22c). Band 2 was fairly prominent at all ages, and in specimens older than two weeks, band 1 often approached or surpassed it quantitatively. The presence of band 3 was dependent upon the magnitude of band 2. In female thoracic muscle LDH patterns, band 2 was relatively prominent while band 3 exhibited very faint staining (Figure 22a). However, in female appendage muscle patterns, band 2 was less prominent than in thoracic muscle while band 3 was more in evidence (Figure 26a). Bands 4 and 5 were prominent in specimens younger than one month, with 5 represented as an inflection point on the leading edge of peak 4 (Figure 22c). The R_f values of the two peaks were constant until about one month when these values displayed considerable variation, or one of the peaks was not represented. If only one peak was present in this vicinity, it was generally prominent and possessed an R_f value corresponding to a mobility midway between 4 and 5. The coalescing

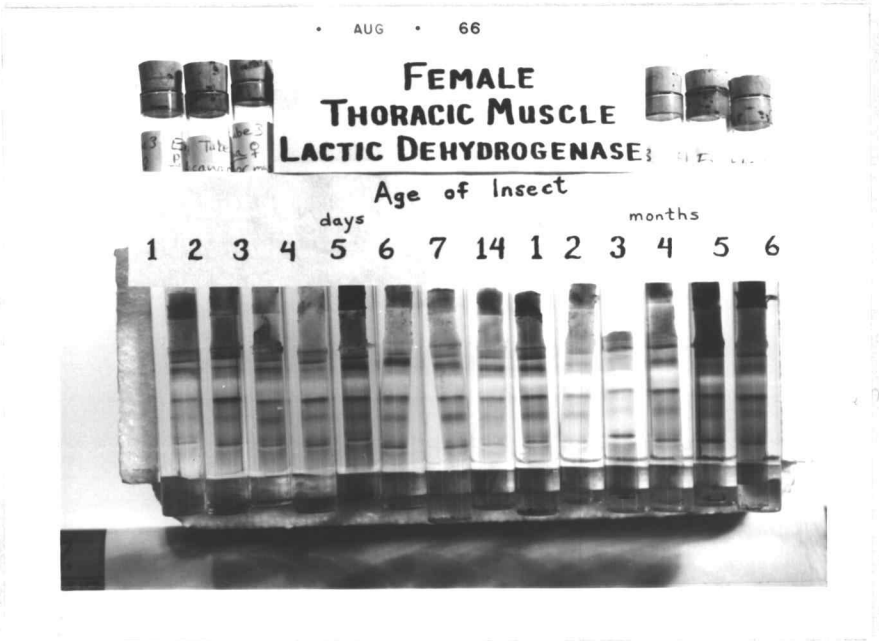


Figure 20. Photograph of female thoracic muscle lactic dehydrogenase gels.

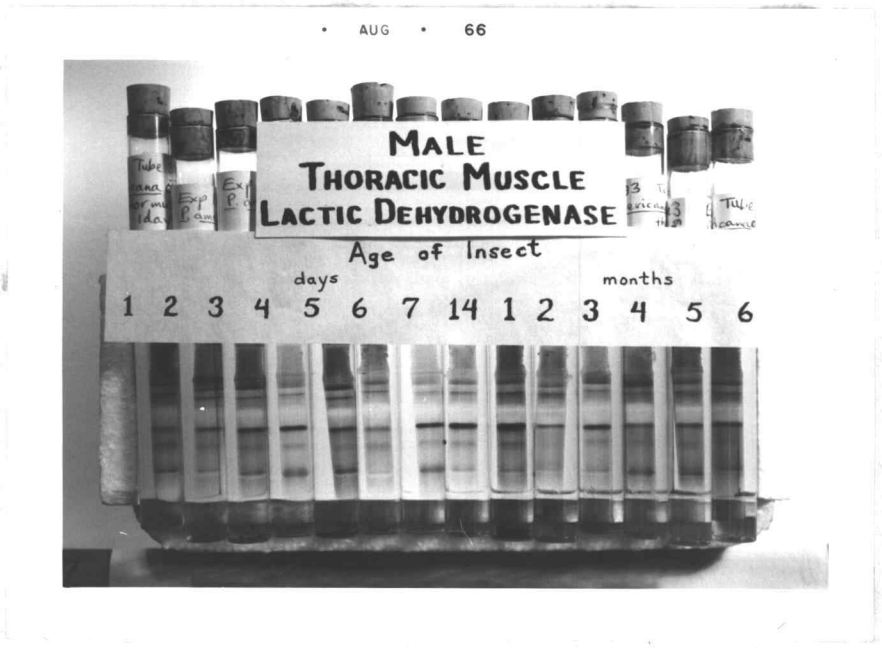
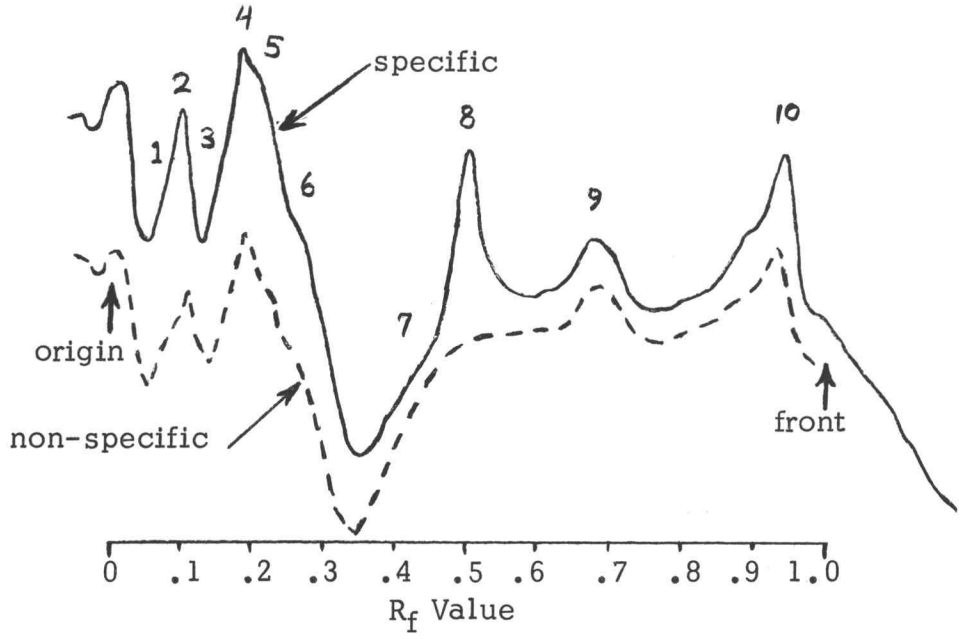
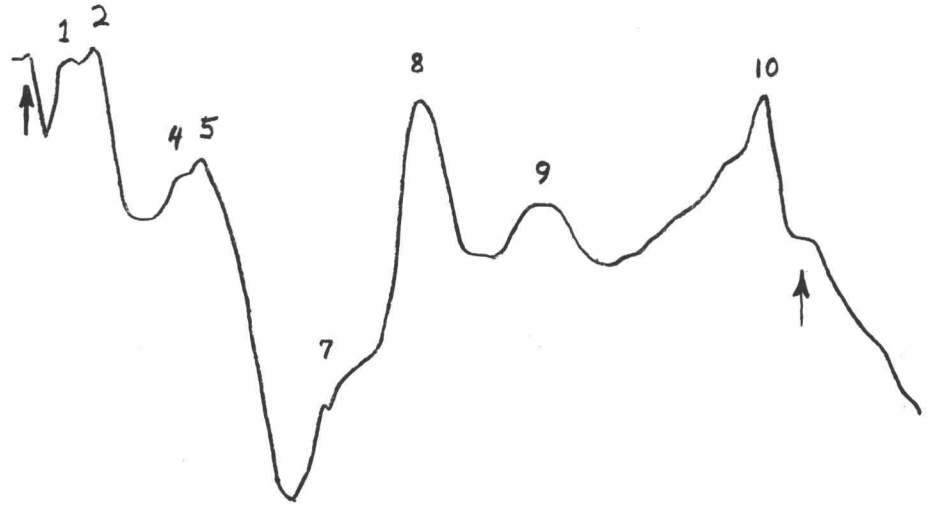


Figure 21. Photograph of male thoracic muscle lactic dehydrogenase gels.



a. one day old specimen



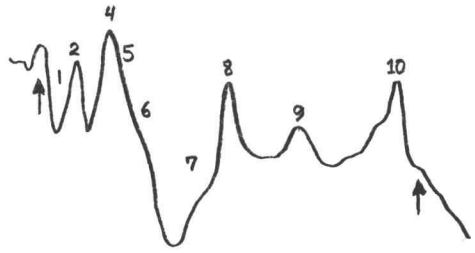
b. six month old specimen

Figure 22. Densitometric scans of female thoracic muscle lactic dehydrogenase.

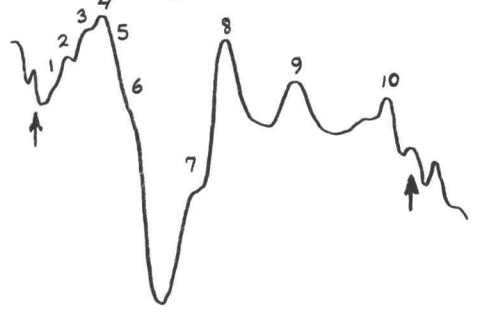
Figure 22. Densitometric scans of female thoracic muscle lactic dehydrogenase.

c. one through 14 day old specimens.

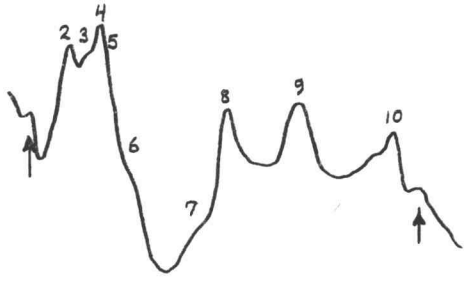
Female Thoracic Muscle Lactic Dehydrogenase



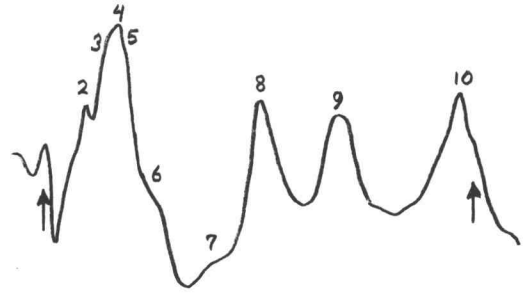
1 day



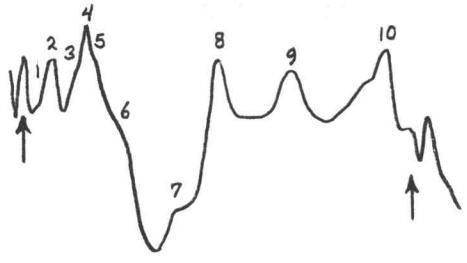
5 day



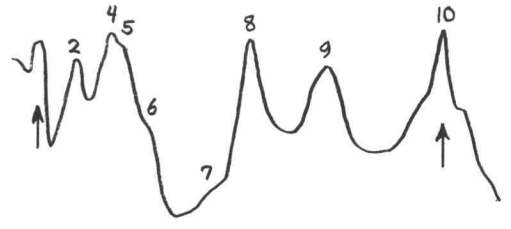
2 day



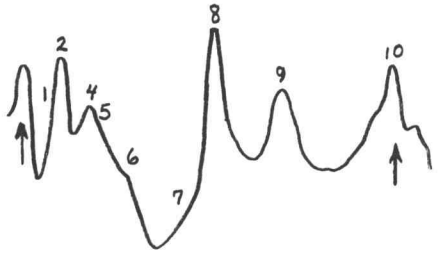
6 day



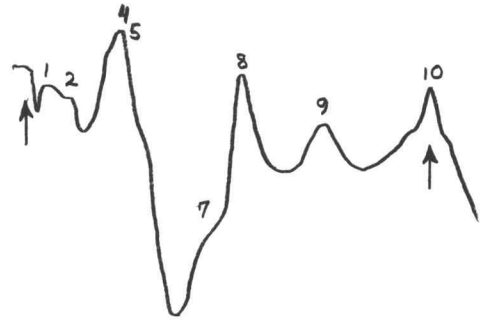
3 day



7 day



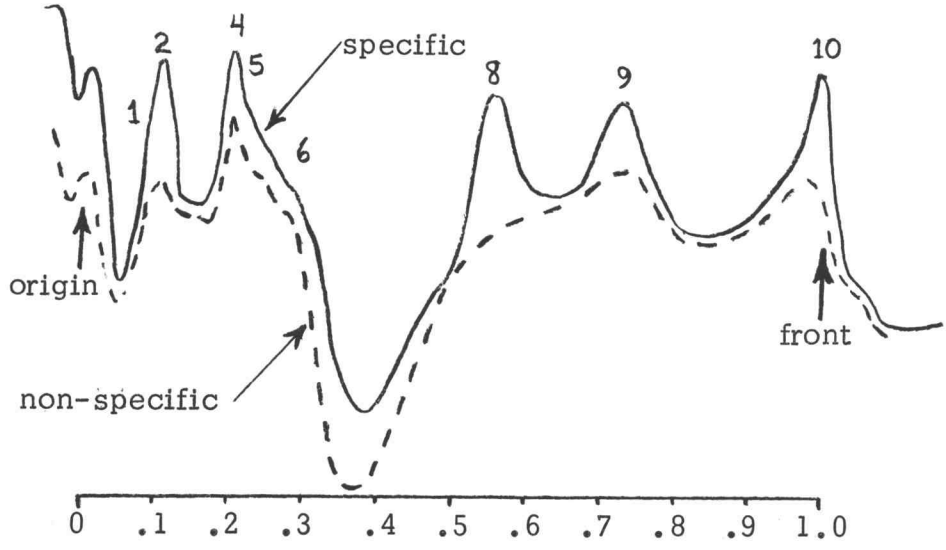
4 day



14 day

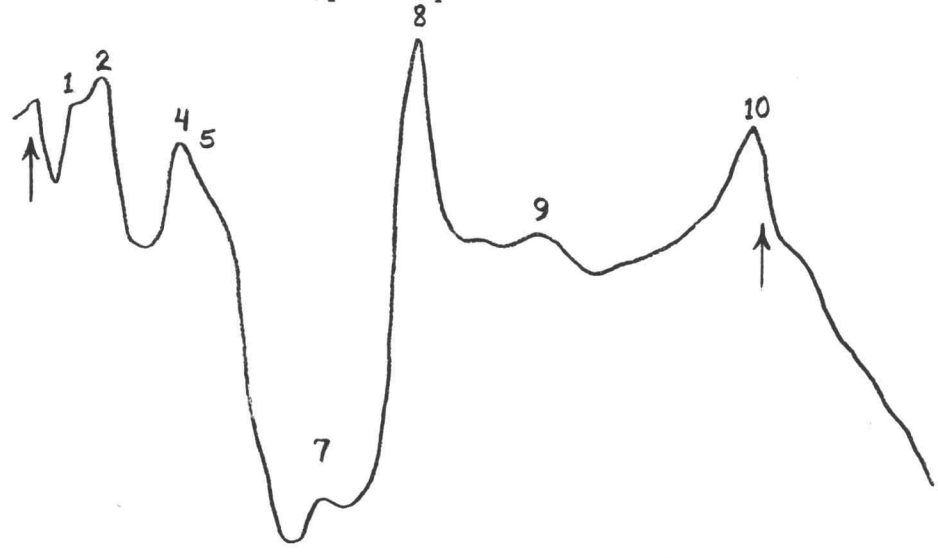
TABLE 11. RELATIVE MOBILITY (R_f) VALUES FOR FEMALE THORACIC MUSCLE LACTIC DEHYDROGENASE

Age	Band Number									
	1	2	3	4	5	6	7	8	9	10
1 day	.057	.094		.18	.208	.265	.416	.498	.681	.947
2 day		.102	.146	.183	.203	.276	.414	.52	.707	.955
3 day	.047	.074	.125	.16	.188	.258	.399	.493	.68	.926
4 day	.066	.112		.186	.207	.282	.447	.521	.703	1.0
5 day	.061	.094	.139	.184	.204	.266	.436	.514	.698	.943
6 day		.094	.146	.167	.186	.258	.396	.502	.698	.97
7 day		.088		.177	.204	.269		.527	.715	1.0
14 day		.065		.160	.183		.395	.486	.692	.955
1 month	.052	.1		.176	.208		.428	.504	.688	.964
2 month	.042	.094		.208		.26	.438	.525	.718	.975
3 month	.038	.068		.162	.192	.282	.387	.519	.706	.97
4 month	.045	.093		.239			.433	.522	.709	.992
5 month	.047	.124		.210	.252	.310	.473	.539	.725	.988
6 month	.055	.099		.210	.237		.429	.538	.704	1.0



R_f Value

a. one day old specimen



b. six month old specimen

Figure 23. Densitometric scans of male thoracic muscle lactic dehydrogenase.

TABLE 12. RELATIVE MOBILITY (R_f) VALUES FOR MALE THORACIC MUSCLE LACTIC DEHYDROGENASE

Age	Band Number									
	1	2	3	4	5	6	7	8	9	10
1 day	.053	.094		.189	.215	.272		.532	.695	.951
2 day		.1	.152	.188	.212	.268		.512	.684	.951
3 day		.092		.199	.227	.271	.438	.538	.725	1.0
4 day		.087		.181	.207	.275	.398	.523	.703	.985
5 day		.068		.172	.196	.228		.496	.704	.940
6 day		.102	.121	.181	.207	.25	.38	.529	.688	.935
7 day		.084		.183	.213	.27		.521	.7	.981
14 day	.057	.084	.145	.186	.206	.247	.392	.514	.692	.95
1 month	.053	.088		.197	.228		.411	.525	.707	.97
2 month	.047	.118		.188	.200	.271	.427	.533	.737	.981
3 month	.049	.093		.179	.205		.384	.534	.683	.974
4 month	.057	.095		.181	.206		.380	.506	.681	.977
5 month	.048	.106		.208	.226	.285	.406	.522	.709	.99
6 month	.049	.090		.200	.225		.408	.531	.698	1.0

of these two bands was more evident in appendage muscle than in thoracic muscle.

Bands 6 and 7 border a curious non-staining clear area in the gel, the identity of which is unknown (Figures 20, 21, 24 and 25). Anderson (1966) noted a similar area in LDH patterns from bacteria attributed to an iron-binding protein which interferes with the staining mechanism. It was extremely difficult to determine whether bands 6 and 7 represented discrete isozymes in the earliest ages and consequently R_f values were recorded if there were inflection points on the electropherogram. The resulting omission of values in these two columns probably is not too significant during these stages. Band 6 was weak or absent and rarely appeared in specimens over one month in age. In specimens older than two weeks, band 7 was more pronounced and was represented by a definite peak on the scan. The older male muscle patterns showed band 7 more distinctly than those of females.

The LDH-specific band 8 was the most prominent and consistent band in the muscle of both sexes. The over-all variation in R_f value of this band was also quite low.

Band 9, another prominent non-specific band, was the previously described orange staining band and was also characterized by low R_f variation. The most rapidly migrating band, number 10, was often represented on the scan by a double or triple peak with the

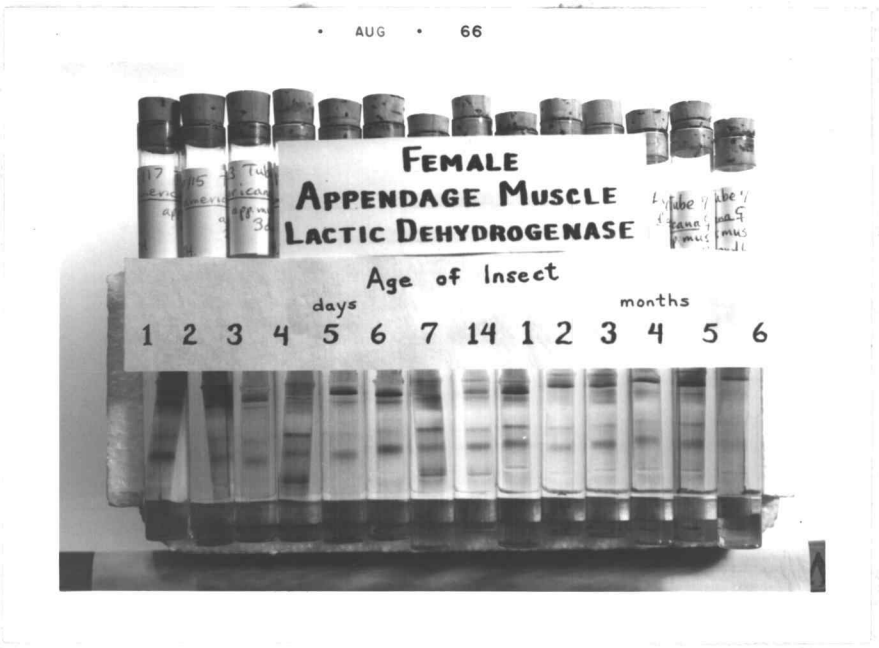


Figure 24. Photograph of female appendage muscle lactic dehydrogenase gels.

Figure 25. Photograph of male appendage muscle lactic dehydrogenase gels.

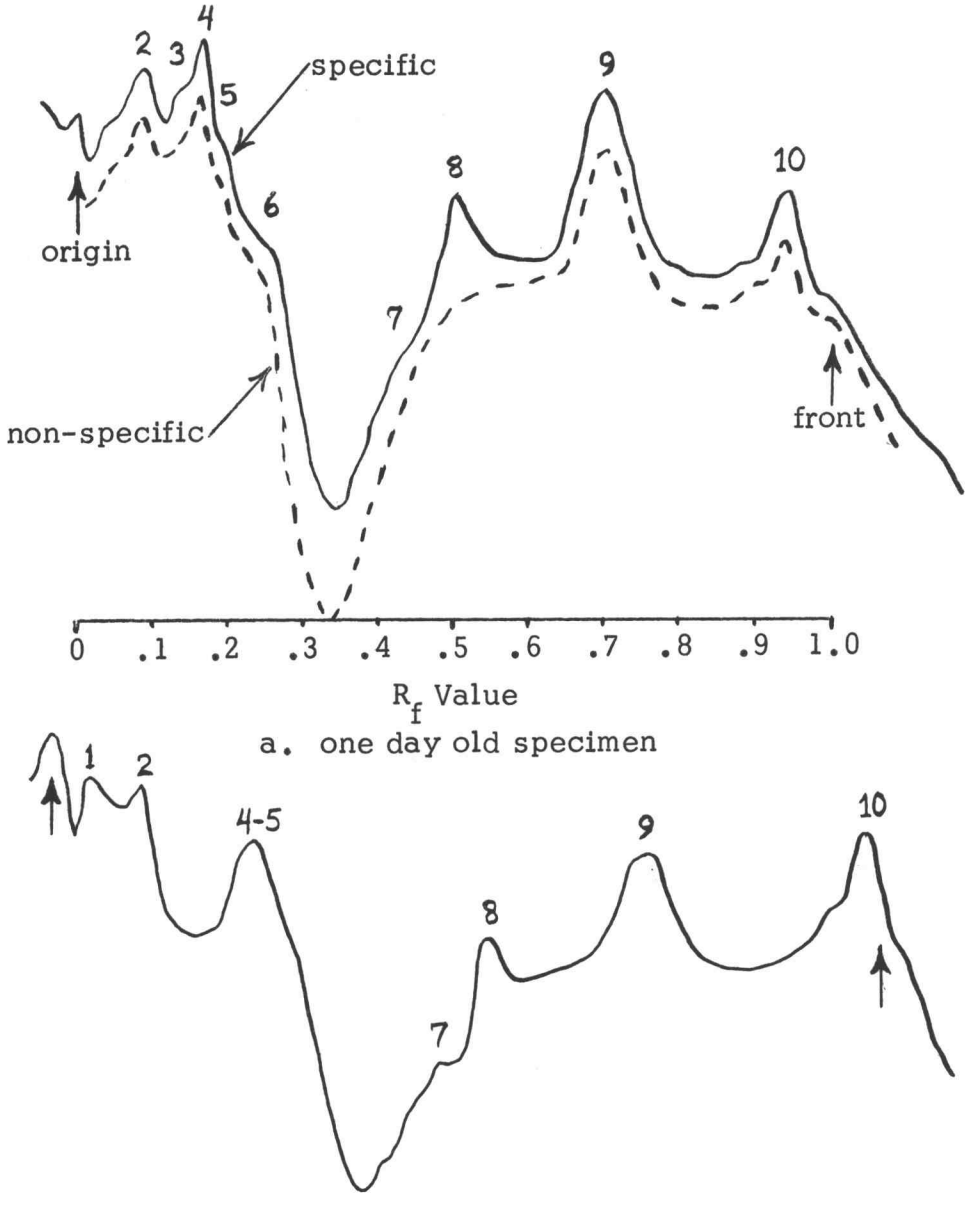
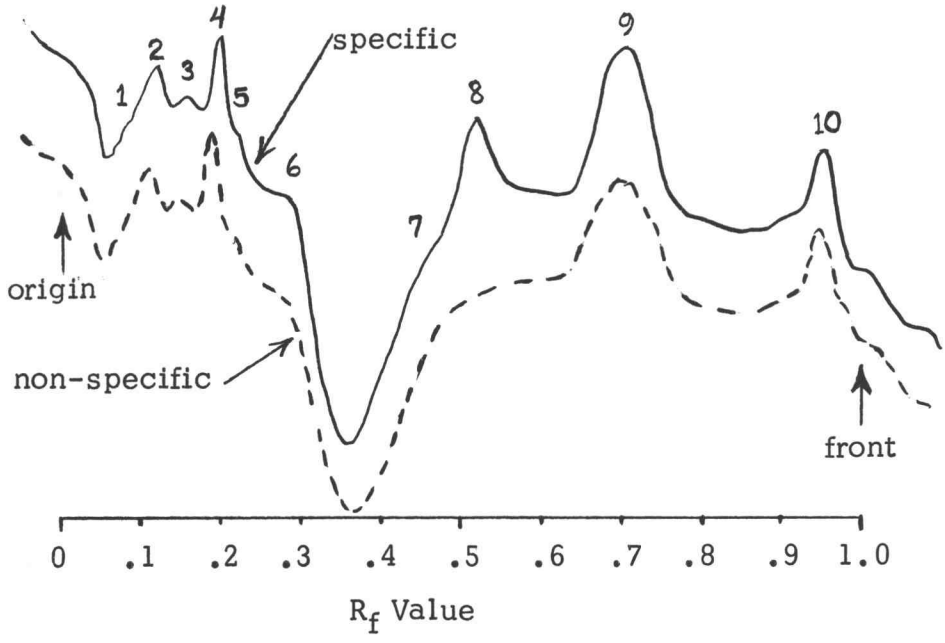


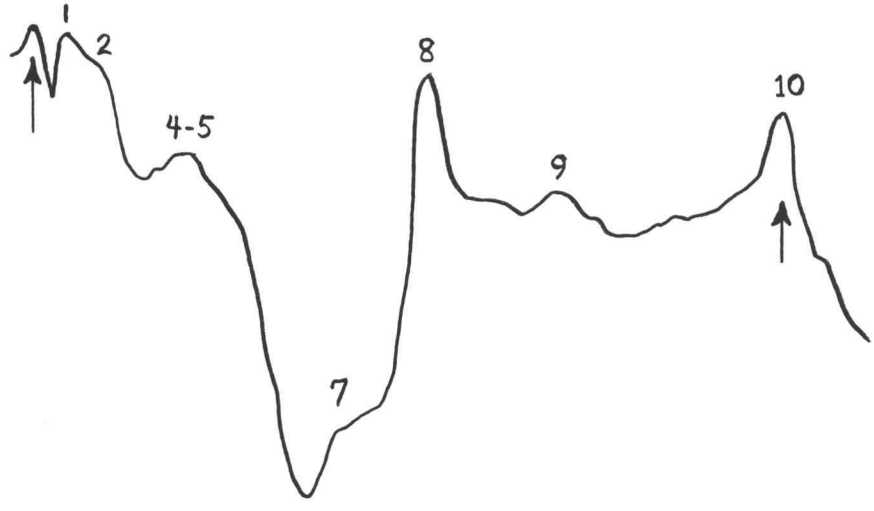
Figure 26. Densitometric scans of female appendage muscle lactic dehydrogenase.

TABLE 13. RELATIVE MOBILITY (R_f) VALUES FOR FEMALE APPENDAGE MUSCLE LACTIC DEHYDROGENASE

Age	Band Number 1	2	3	4	5	6	7	8	9	10
1 day	.053	.094	.14	.17	.196		.419	.498	.702	.932
2 day		.083	.132	.162	.18	.256	.395	.493	.684	.932
3 day		.088	.134	.164	.195	.241	.412	.496	.691	.94
4 day		.089	.115	.173	.204	.265		.5	.685	.973
5 day	.034	.097	.134	.183	.198	.261	.436	.515	.727	.933
6 day		.1		.171	.2	.246		.496	.68	.925
7 day		.095	.119	.175	.199	.267	.415	.51	.705	.987
14 day		.097		.155	.186	.24		.488	.633	.942
1 month	.051	.133		.185	.206		.429	.468	.708	.91
2 month	.052	.12		.157	.221		.42	.532	.73	.973
3 month	.051	.106		.169	.196	.279	.439	.534	.722	.98
4 month	.053	.110		.227			.428	.515	.701	.977
5 month	.038	.115		.222		.291		.529	.742	.988
6 month	.043	.107		.246			.466	.53	.726	.995



a. one day old specimen



b. six month old specimen

Figure 27. Densitometric scans of male appendage muscle lactic dehydrogenase.

TABLE 14. RELATIVE MOBILITY (R_f) VALUES FOR MALE APPENDAGE MUSCLE LACTIC DEHYDROGENASE

Age	Band Number 1	2	3	4	5	6	7	8	9	10
1 day	.058	.093	.13	.171	.197	.26	.42	.502	.691	.955
2 day	.058	.092	.142	.177	.204	.242	.427	.512	.7	.95
3 day	.04	.083	.138	.171	.193	.244	.433	.509	.702	.965
4 day		.076		.178	.201	.273	.436	.5	.681	.953
5 day	.049	.118		.191	.215	.252	.402	.5	.69	.926
6 day		.089		.171	.201	.268	.409	.509	.699	.976
7 day		.095		.181	.204	.273	.435	.52	.704	.99
14 day		.085		.178	.205	.259	.402	.502	.688	.961
1 month	.042	.077		.188	.211	.261	.398	.521	.716	.972
2 month	.059	.106		.212		.27	.396	.557	.722	.98
3 month	.046	.087		.205	.235		.436	.538	.74	.994
4 month	.04	.066		.179			.365	.496	.675	.978
5 month	.05	.104		.223			.434	.519	.711	.989
6 month	.047	.078		.202			.399	.531	.702	1.0

largest peak representing band 10. Upon examination of the gel, it could be determined that the bands on either side of band 10 were less dense and stained a light blue rather than the purple tint indicative of dehydrogenase activity. The blue color suggests that there may be an interaction between the bromphenol blue and this fast migrating non-specific species.

Gilbert and Goldberg (1966) found seven bands of LDH activity in adult ovary, six in embryo and two in fat body. The most rapidly migrating of the LDH isozymes in each of these tissues had a .34 R_f value which may correspond to the R_f value of the LDH-specific band found in Periplaneta muscle. Apparently LDH muscle patterns were not examined in Leucophaea, although a pattern with one band was recorded for muscle GPDH. Because of similar R_f values, there may be a possible relationship between the most rapidly migrating LDH band in Leucophaea tissues and the muscle LDH band in Periplaneta.

The pattern displayed by lactic dehydrogenase in muscle homogenates of Periplaneta proved to be very stable among individuals of the same and different ages. Therefore, it may prove to have value as a phyletic indicator at higher taxa levels because of its apparent stability at the species level.

Muscle α-glycerophosphate Dehydrogenase Patterns

As with the other muscle dehydrogenases, α-glycerophosphate

dehydrogenase patterns were characterized by non-specific activity in the absence of substrate. Because of equal incubation intervals, the no-substrate control was almost identical for LDH and GPDH. Therefore, the non-specific bands were comparable. The distinguishing factor was the appearance of one (8) and occasionally two (8 and 9) GPDH-specific bands on the gel (Figure 10 and 11).

There was a degree of quantitative and qualitative variation associated with the appearance of GPDH-specific bands in muscle. The variation was more pronounced in the patterns of female thoracic muscle where the GPDH-specific bands were usually weak or absent (Figure 28). Replication of female thoracic muscle specimens of identical age occasionally revealed patterns both with and without GPDH-specific bands (Table 15 and Figure 30c). In Figure 30c, a light and a heavy traced scan for specimens aged one, five and 14 days indicates that both patterns were encountered for that age interval during the course of investigation. Female appendage muscle and male thoracic and appendage muscle were all typified by strongly staining GPDH-specific band 8 or bands 8 and 9. There appeared to be no relationship between the density of these main GPDH-specific bands and increasing age of the specimen.

As previously stated, the GPDH non-specific bands were practically identical to those reported under the section covering muscle LDH. The only variation from LDH patterns was a few

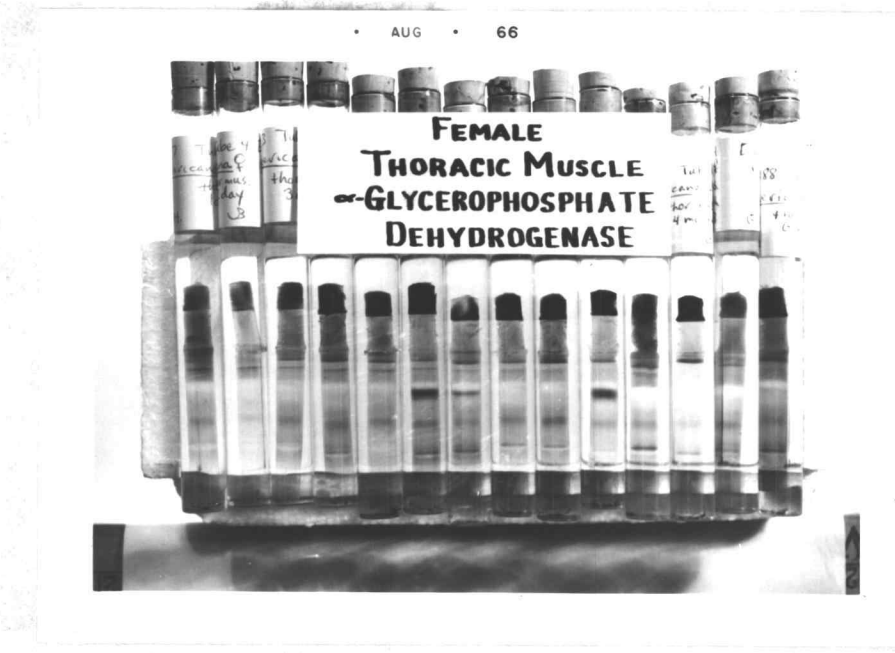


Figure 28. Photograph of female thoracic muscle α-glycerophosphate dehydrogenase gels.

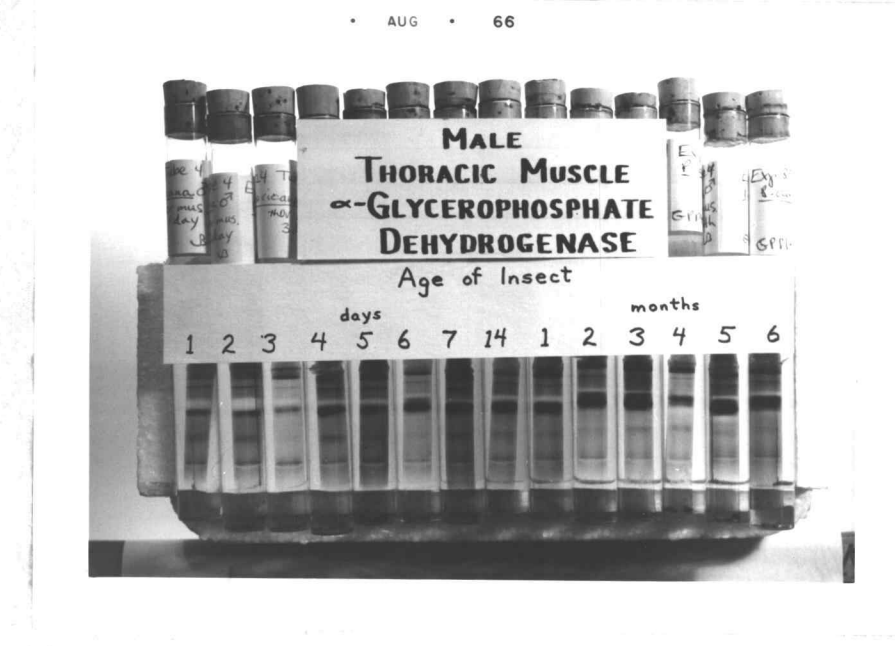
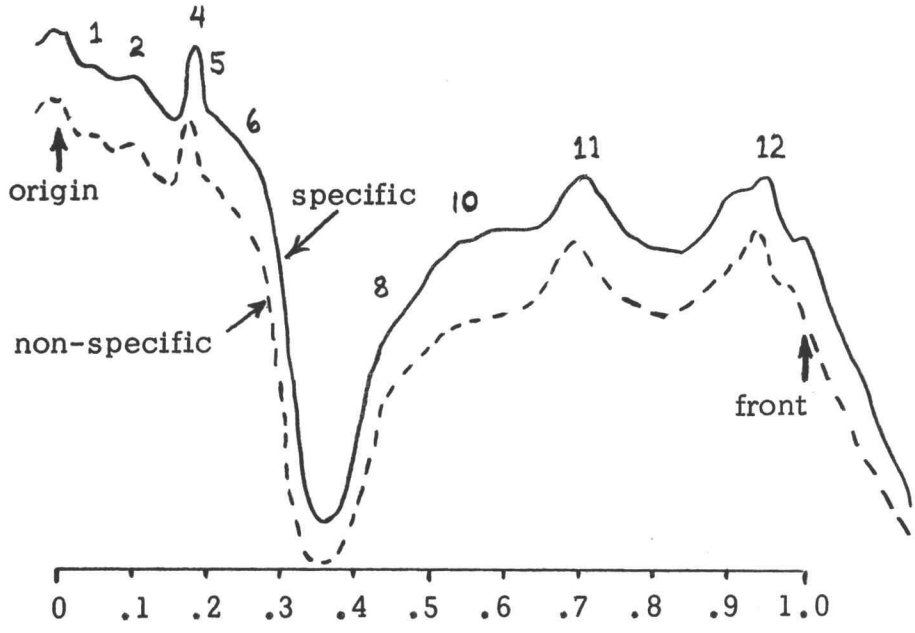
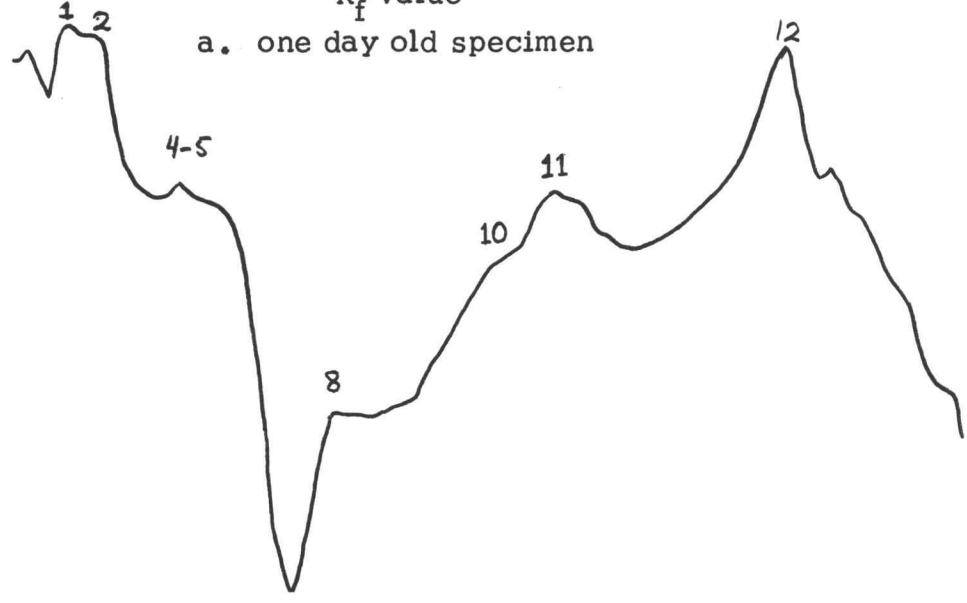


Figure 29. Photograph of male thoracic muscle α-glycerophosphate dehydrogenase gels.



a. one day old specimen



b. six month old specimen

Figure 30. Densitometric scans of female thoracic muscle α-glycerophosphate dehydrogenase.

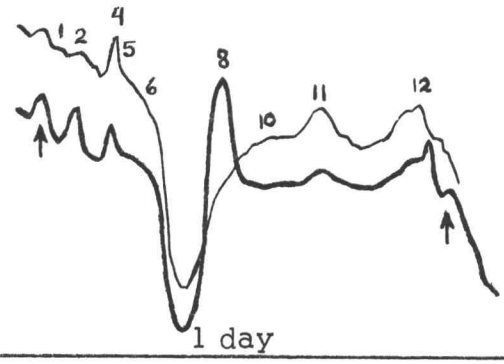
Figure 30. Densitometric scans of female thoracic muscle a-glycerophosphate dehydrogenase.

c. one through 14 day old specimens.

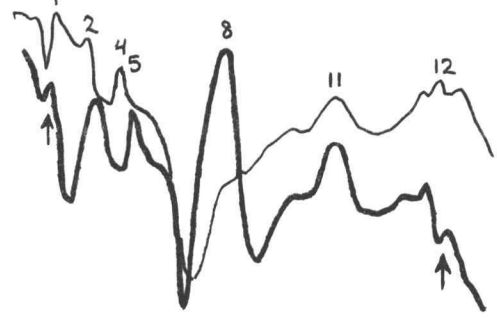
Figure 30. Densitometric scans of female thoracic muscle a-glycerophosphate dehydrogenase.

c. one through 14 day old specimens.

Female Thoracic Muscle α-glycerophosphate Dehydrogenase

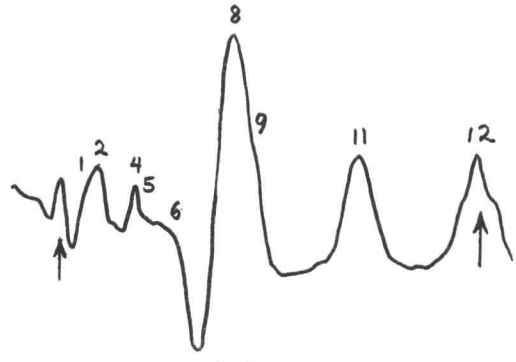


1 day

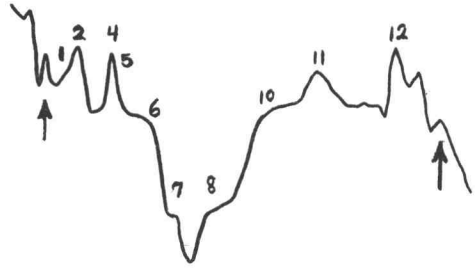


5 day

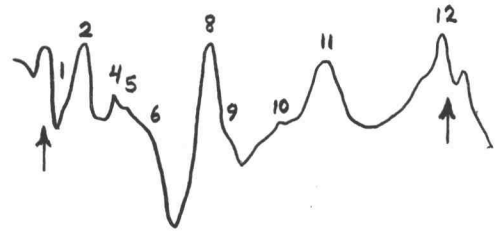
2 day



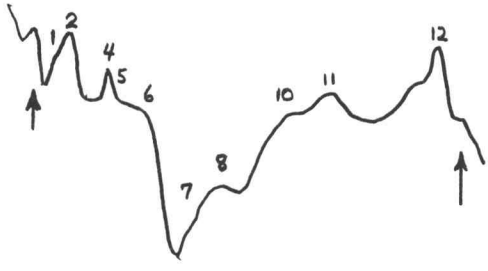
6 day



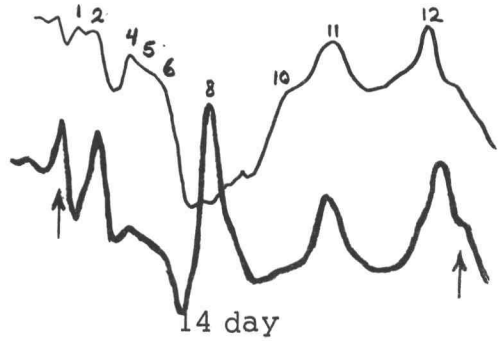
3 day



7 day



4 day



14 day

TABLE 15. RELATIVE MOBILITY (R_f) VALUES FOR FEMALE THORACIC MUSCLE α-GLYCEROPHOSPHATE DEHYDROGENASE

Age	Band Number 1	2	3	4	5	6	7	8	9	10	11	12	Density of Bands 8-9
1 day	.039	.092		.177	.2	.254		.427		.527	.689	.931	light
2 day	.052	.112		.184	.212	.254	.34	.428		.588	.696	.952	light
3 day	.038	.08		.164	.187	.252	.315	.431		.554	.675	.92	light
4 day	.047	.084		.172	.193	.256	.351	.441		.602	.705	.956	light
5 day		.076		.171	.201	.262	.315	.46		.585	.692	.95	light
6 day	.051	.088		.175	.197	.237		.408	.456		.708	.99	heavy
7 day	.053	.091		.175	.202	.274		.403	.456	.59	.696	.98	heavy
14 day	.052	.092		.188	.204	.244	.356	.472		.596	.708	.96	light
1 month	.052	.096		.2	.22	.283	.386	.488		.616	.72	.992	light
2 month	.074	.137		.248		.31		.452		.642	.741	.993	heavy
3 month	.044	.188		.228		.304		.496		.644	.736	.996	light
4 month	.037	.082		.197			.372	.439	.498	.603	.688	.974	light
5 month	.066	.124		.236		.301		.514		.645	.741	1.0	light
6 month	.046	.076		.190		.259		.403		.6	.688	.992	light
1 day	.052	.082		.164	.187			.437			.679	.933	heavy
5 day		.129		.22	.243	.286		.439		.631	.725	.96	heavy
14 day	.055	.094		.165	.188			.377	.443	.53	.675	.969	heavy
5 month	.046	.112		.215				.438	.503	.638	.700	.965	heavy

non-specific bands which might be present or masked depending upon the dominance of the GPDH-specific band. The clear non-staining zone described under muscle LDH was also present in GPDH patterns. This clear zone was not as extensive as that found for LDH because the leading edge was bordered by the more slowly migrating GPDH-specific band. The non-specific band 7 seen on LDH gels was masked by the GPDH-specific band 8 and was only apparent when the specific band was weak or absent. Band 10, which was probably masked by the LDH-specific band 8, was more distinct on both non-specific and GPDH-specific patterns. Bands 11 and 12 appeared as they occurred for LDH patterns.

Gilbert and Goldberg (1966) found strong GPDH activity only in muscle extracts but a small amount was present in fat body preparations. The single band of activity in Leucophaea muscle GPDH had an R_f value of 0.48 which compares quite closely to an average R_f value for the main band of Periplaneta muscle GPDH. Although this main GPDH band in Periplaneta displayed considerable variation in its density and R_f value, it may be comparable to the main band in Leucophaea.

Generally, the unstable nature of GPDH band 8 or 8 and 9 would indicate a possible thermo-labile isozyme with the female thoracic muscle possessing the most temperature sensitive isozyme. The density of this main band may also be dependent on temperature

although every attempt was made to keep the tissue homogenate cold during the analysis. Nevertheless, variation which this enzyme expresses makes it, like the esterases, difficult to employ in a biochemical systematical study.

The usual relationship of low LDH and high GPDH in thoracic muscle and the converse for appendage muscle was not demonstrated in Periplaneta. All Periplaneta muscle LDH and GPDH isozyme patterns displayed approximately the same density when the sample volumes were equivalent and incubated for the same period of time. For Leucophaea, Gilbert and Goldberg (1966) showed that when an active LDH system was present, the activity of GPDH was low or absent and vice versa. The absence of such a correlation in the related genus, Periplaneta, suggests that this relationship between LDH and GPDH is not universal.

Hemolymph Esterase Patterns

The immense difference that exists between hemolymph and muscle enzyme patterns prompted the discussion of their diversity in this separate section. It would be extremely difficult to indicate interrelationships between two bands found on hemolymph and muscle patterns without the assistance of immunoelectrophoresis. Therefore, in this section only similarities and differences between male and female hemolymph patterns for each specific enzyme will

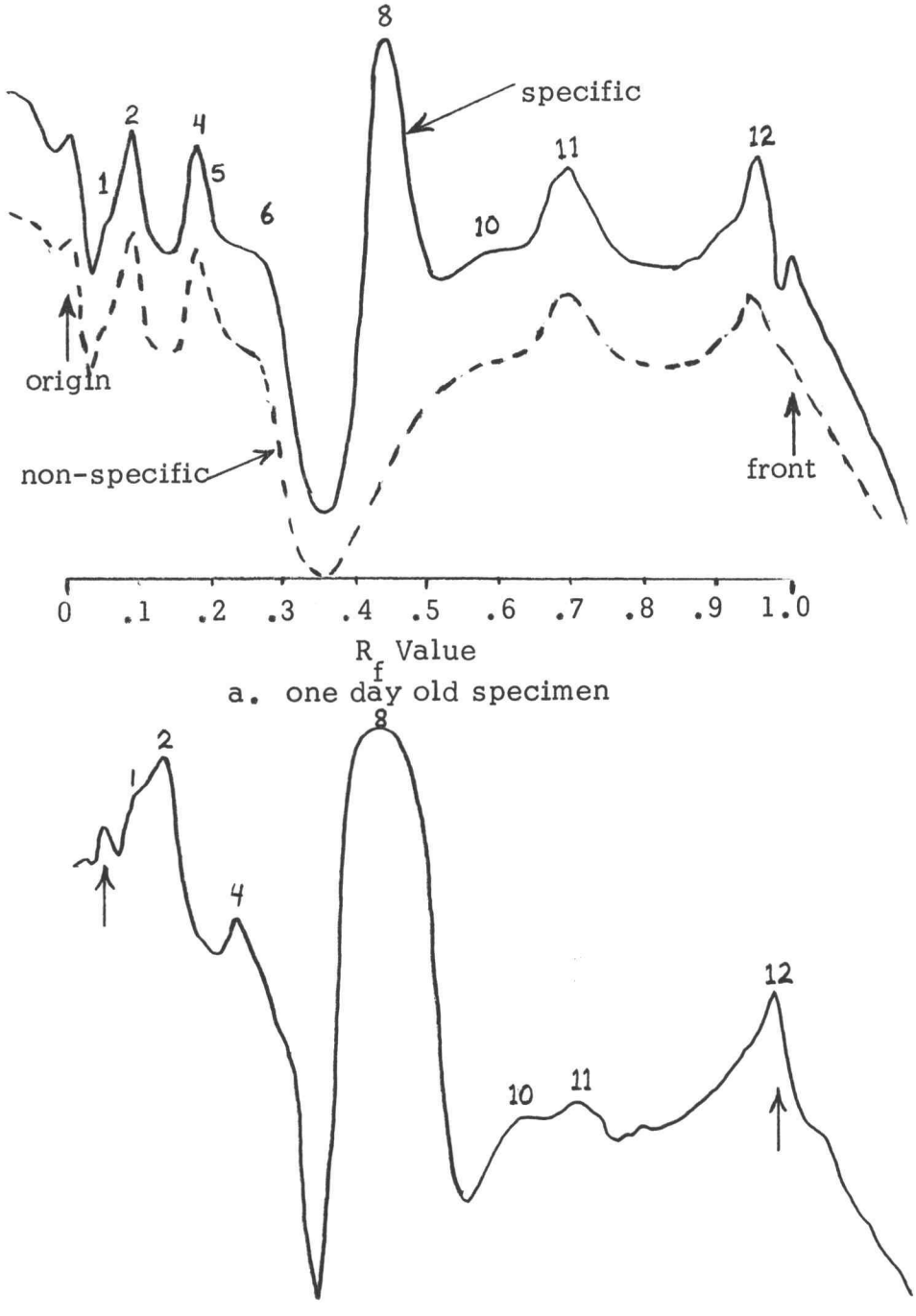


Figure 31. Densitometric scans of male thoracic muscle α-glycerophosphate dehydrogenase.

TABLE 16. RELATIVE MOBILITY (R_f) VALUES FOR MALE THORACIC MUSCLE α-GLYCEROPHOSPHATE DEHYDROGENASE

Age	Band Number 1	2	3	4	5	6	7	8	9	10	11	12	Density of Bands 8-9
1 day	.05	.08		.172	.199	.26		.431		.57	.687	.946	heavy
2 day	.054	.073		.173	.204	.238		.439			.689	.939	heavy
3 day		.059		.17	.194	.245		.395	.454		.691	.948	heavy
4 day	.053	.082		.172	.201			.41			.691	.946	heavy
5 day	.048	.08		.181	.205	.253		.402			.695	.936	heavy
6 day	.056	.1		.181	.215	.259		.389	.444		.7	.945	heavy
7 day	.042	.084		.192	.215	.26		.379			.663	.985	heavy
14 day		.075		.188	.212			.424			.698	.953	heavy
1 month	.023	.073		.183	.211	.26		.427		.585	.703	.971	heavy
2 month	.051	.095		.194	.23			.418	.442	.583	.719	.981	heavy
3 month	.034	.091		.185				.385	.430		.683	.970	heavy
4 month	.033	.07		.148	.218	.256		.348	.393	.537	.641	.944	heavy
5 month		.095		.202	.24	.273		.446		.636	.707	.971	heavy
6 month	.041	.086		.200				.408		.620	.702	.992	heavy

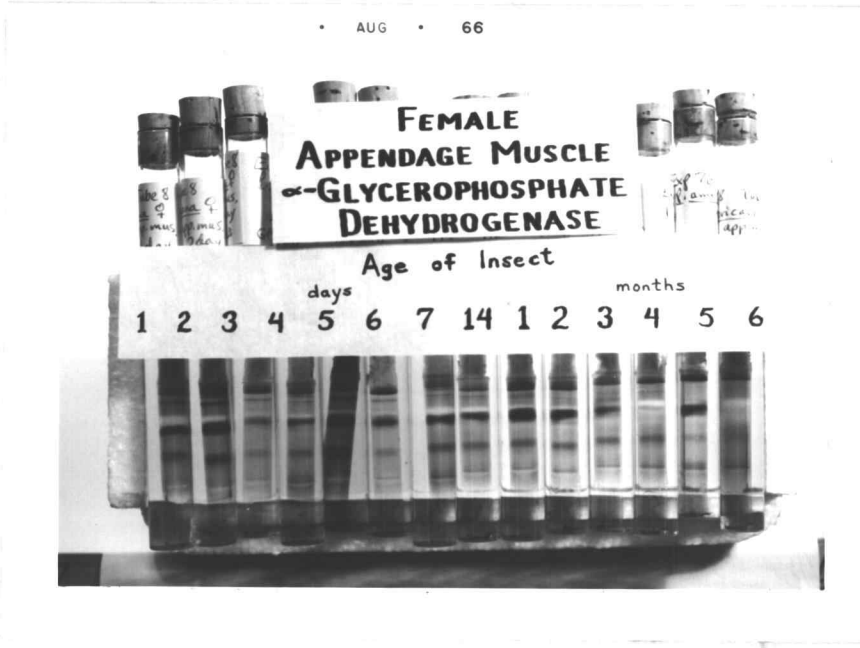


Figure 32. Photograph of female appendage muscle α-glycerophosphate dehydrogenase gels.

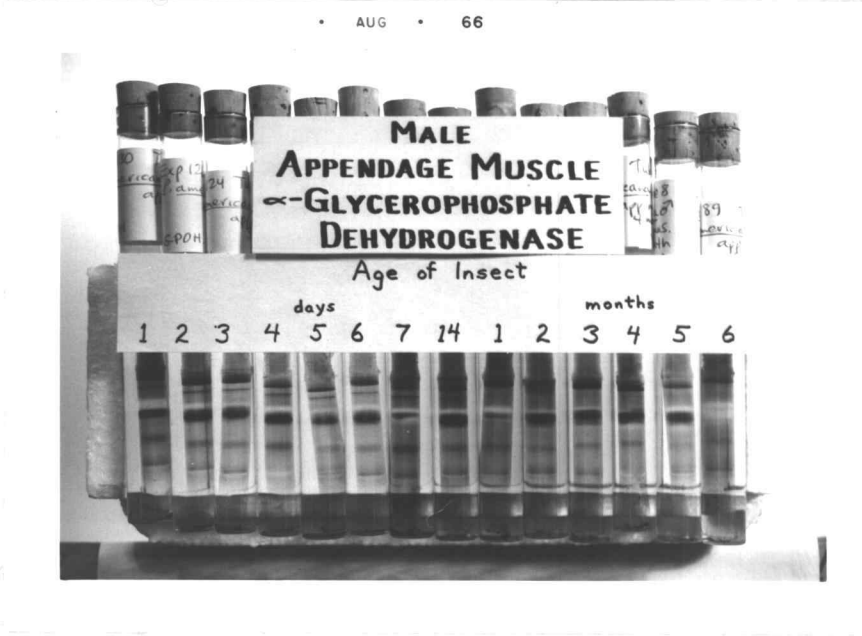
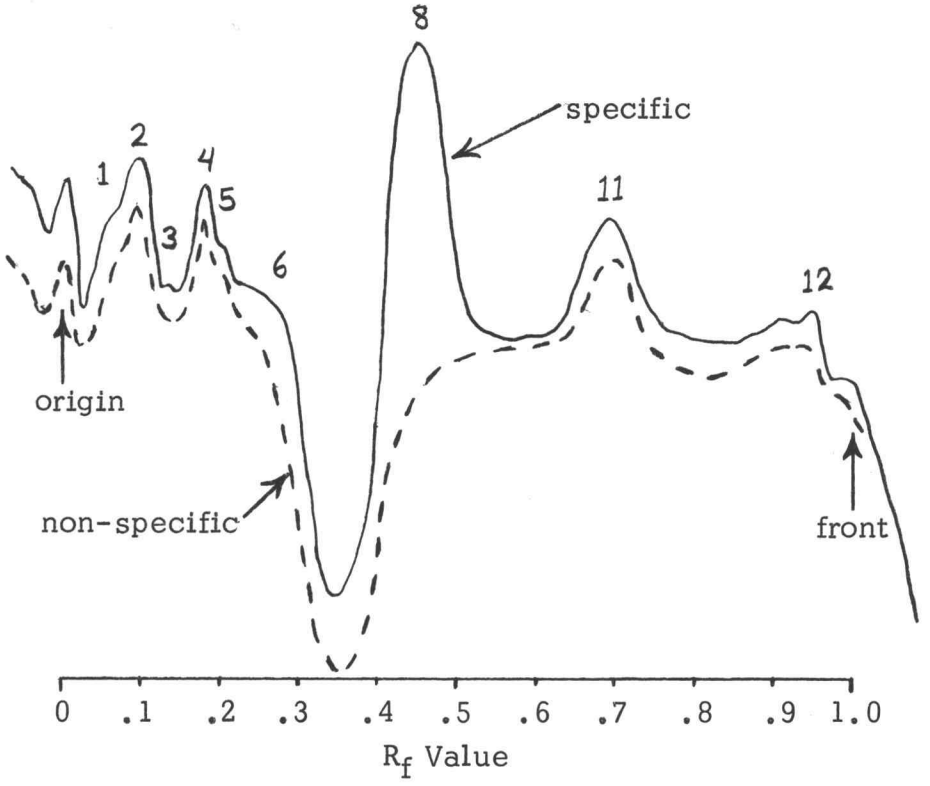
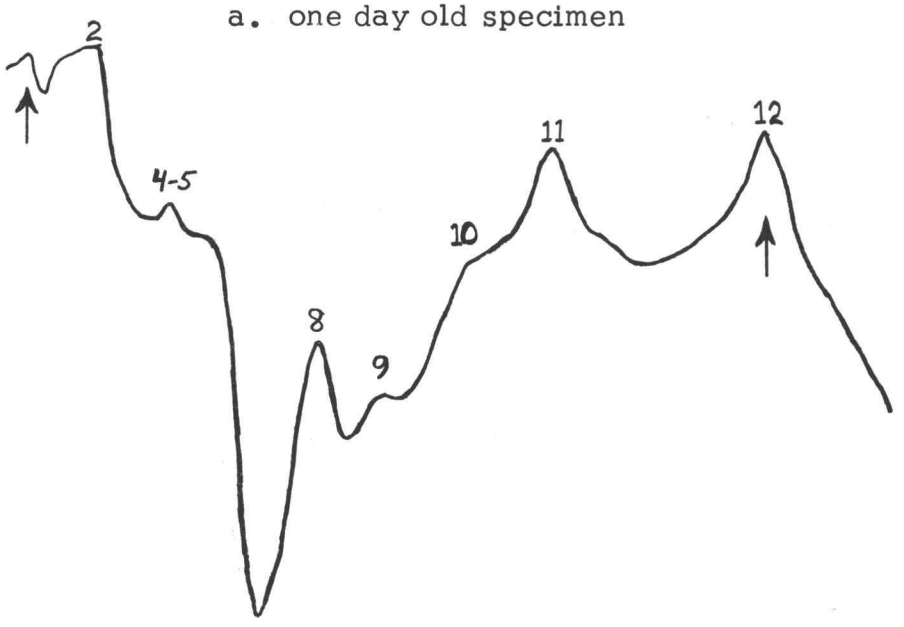


Figure 33. Photograph of male appendage muscle α-glycerophosphate dehydrogenase gels.



a. one day old specimen

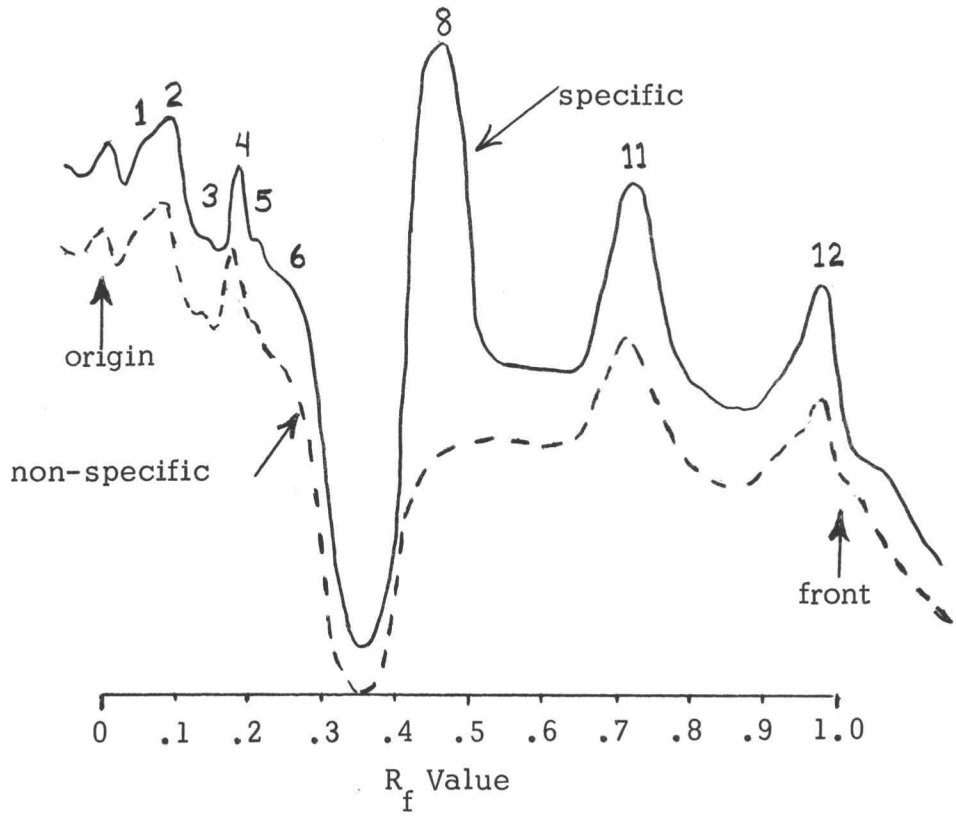


b. four month old specimen

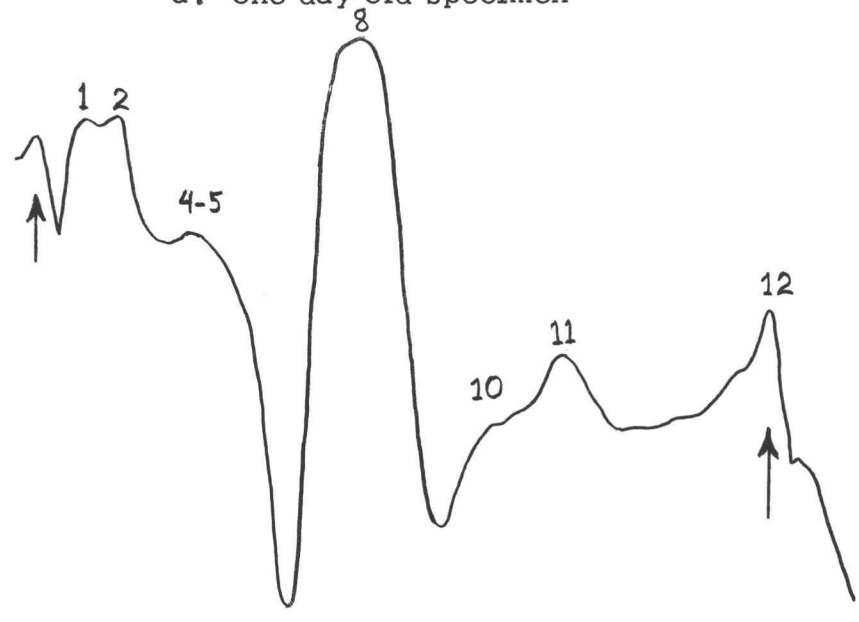
Figure 34. Densitometric scans of female appendage muscle α-glycerophosphate dehydrogenase.

TABLE 17. RELATIVE MOBILITY (R_f) VALUES FOR FEMALE APPENDAGE MUSCLE α-GLYCEROPHOSPHATE DEHYDROGENASE

Age	Band Number 1	2	3	4	5	6	7	8	9	10	11	12	Density of Bands 8-9
1 day	.054	.087	.123	.17	.196	.261		.438		.583	.681	.931	heavy
2 day	.061	.083	.112	.181	.206	.245		.386	.426		.704	.95	heavy
3 day	.049	.083		.17	.196	.253		.385	.445	.558	.701	.946	heavy
4 day	.061	.088	.114	.179	.21	.271		.381	.461	.555	.705	.99	heavy
5 day	.048	.104	.141	.185	.211	.252		.419		.585	.707	.922	heavy
6 day	.054	.092	.138	.173	.2	.25		.369	.396		.692	.935	heavy
7 day	.073	.096	.123	.181	.204	.262		.42		.573	.708	.988	heavy
14 day	.063	.117		.192	.218	.264		.41		.599	.724	.987	heavy
1 month	.054	.075	.113	.179	.208			.412		.6	.716	.99	heavy
2 month	.063	.111		.218				.38	.466	.577	.711	.968	heavy
3 month	.036	.099		.201				.379		.565	.7	.965	heavy
4 month	.06			.17				.348	.46	.576	.668	.960	heavy
5 month		.076		.158	.191			.36		.533	.64	.93	heavy
6 month		.098		.212	.247			.435			.690	1.0	light



a. one day old specimen



b. five month old specimen

Figure 35. Densitometric scans of male appendage muscle α-glycerophosphate dehydrogenase.

TABLE 18. RELATIVE MOBILITY (R_f) VALUES FOR MALE APPENDAGE MUSCLE α-GLYCEROPHOSPHATE DEHYDROGENASE

Age	Band Number 1	2	3	4	5	6	7	8	9	10	11	12	Density of Bands 8-9
1 day	.043	.082	.133	.176	.203	.258		.45			.708	.965	heavy
2 day	.058	.088		.177	.204	.258		.419			.708	.957	heavy
3 day	.048	.088		.173	.199	.254		.386	.419		.702	.963	heavy
4 day	.043	.086		.167	.191	.241		.389	.428		.7	.945	heavy
5 day	.046	.097		.174	.205	.247		.355	.409	.552	.683	.888	heavy
6 day	.039	.083	.122	.176	.197	.248		.376	.419		.685	.932	heavy
7 day	.052	.1		.194	.220	.254		.366		.57	.698	.951	heavy
14 day	.067	.107		.178	.217	.265		.417			.715	.980	heavy
1 month	.049	.076		.186	.205	.27		.403	.486	.59	.711	.97	heavy
2 month	.04	.093		.214				.408	.493	.617	.726	.976	heavy
3 month	.039	.098		.217				.433	.5	.59	.740	.996	heavy
4 month	.039	.144		.216				.352		.568	.648	.964	heavy
5 month	.064	.116		.216				.452		.632	.782	1.0	heavy
6 month	.048	.076		.199				.371	.494	.59	.705	1.0	light

be stressed.

Hemolymph esterases were characterized by three main consistently appearing species which exhibited low migration. Band 6 always predominated in both sexes with the smaller peak representing band 2 usually exceeding peak 4 (Figures 38a, b and 39a, b). Peak 4 generally decreased in size with increasing age while aging had a negligible effect upon peaks 2 and 6.

The slowly migrating minor bands were represented by fairly stable R_f values. Band 1 initially was represented by an inflection point on the trailing edge of peak 2 but in specimens older than one month, it was often represented by a discrete peak. The weak bands 3 and 5 were not consistent in their appearance. The R_f value for band 3 varied initially for male as compared to female specimens implying that it may not be the same band in both sexes (Tables 19 and 20). The minor band 5 seldom appeared.

Bands 7 through 15 were highly variable qualitatively and quantitatively. There was no correlation between age or sex and their appearance. However, it was observed that females and males sampled on the same day usually gave very similar patterns. This suggests a possible unknown variable in technique which may have been overlooked. In addition, the close proximity of bands 7 through 15 gave rise to problems in identity. If a slight discrepancy existed with respect to the R_f values of two corresponding bands between a male

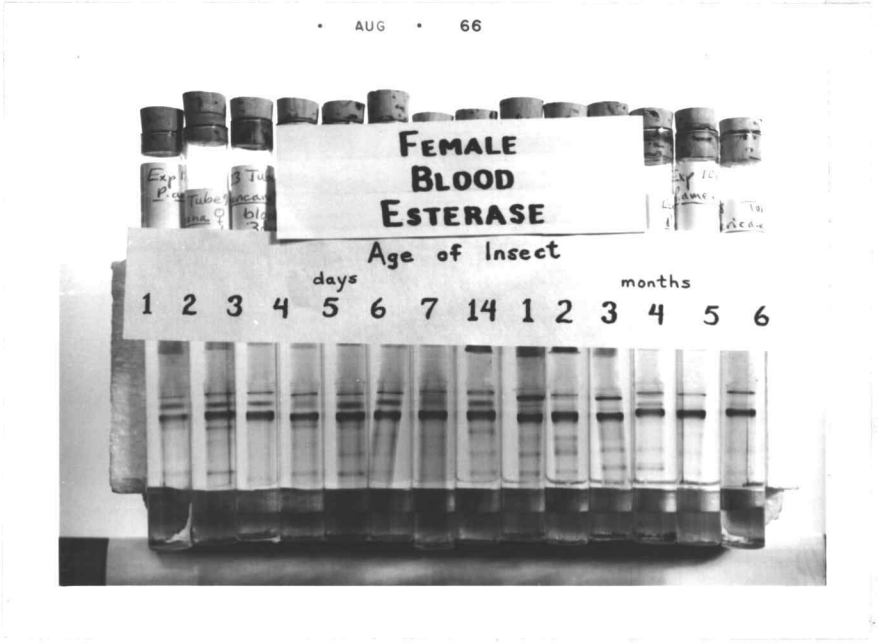


Figure 36. Photograph of female hemolymph esterase gels.

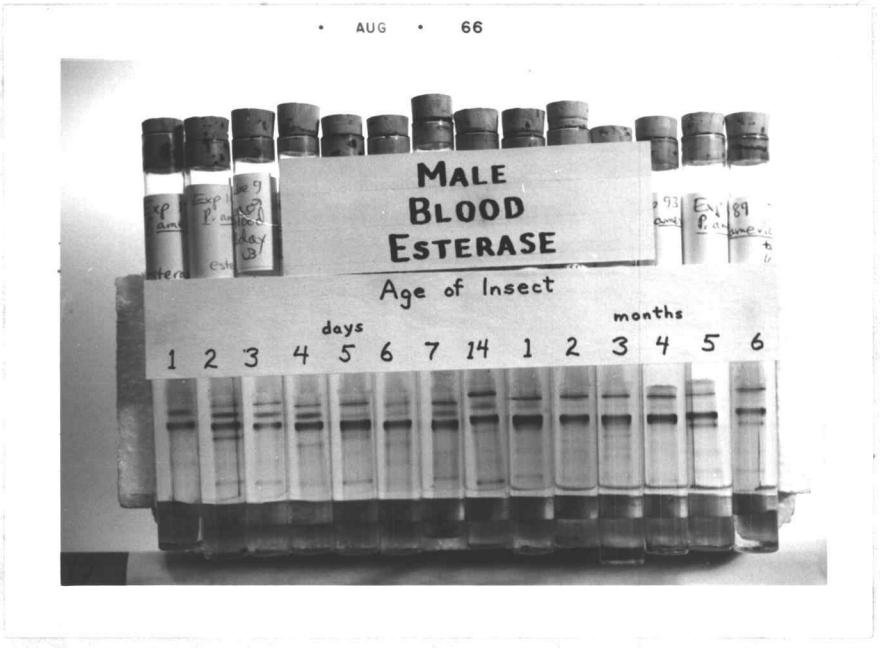
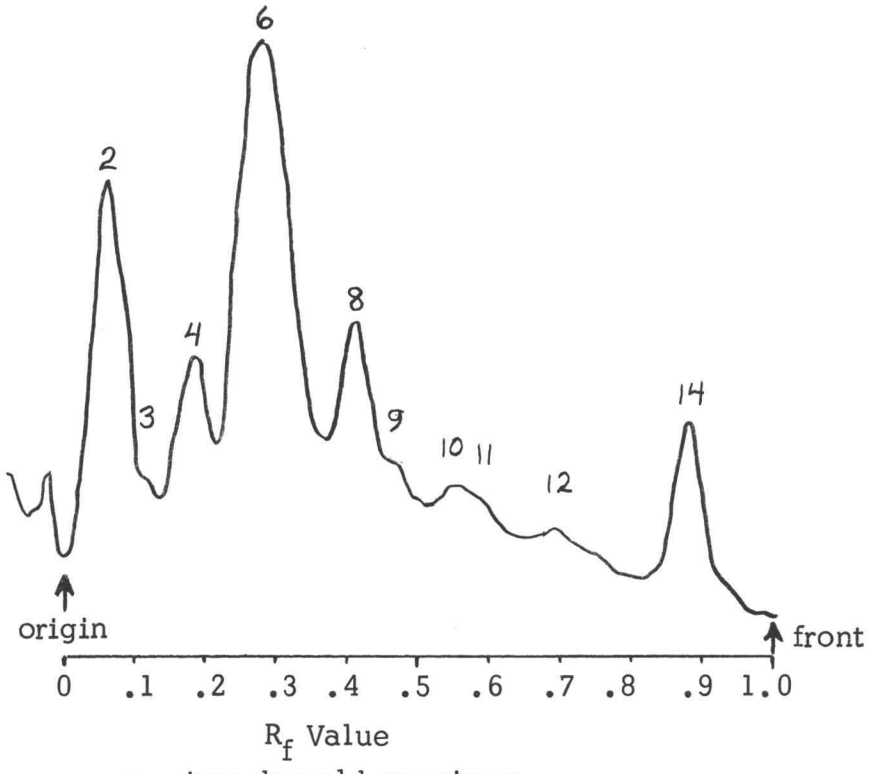
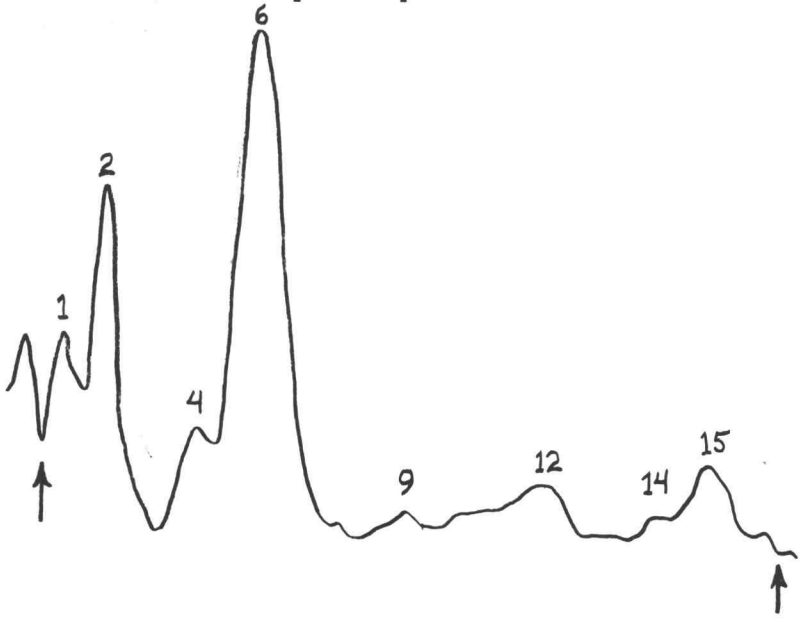


Figure 37. Photograph of male hemolymph esterase gels.



a. two day old specimen



b. six month old specimen

Figure 38. Densitometric scans of female hemolymph esterase.

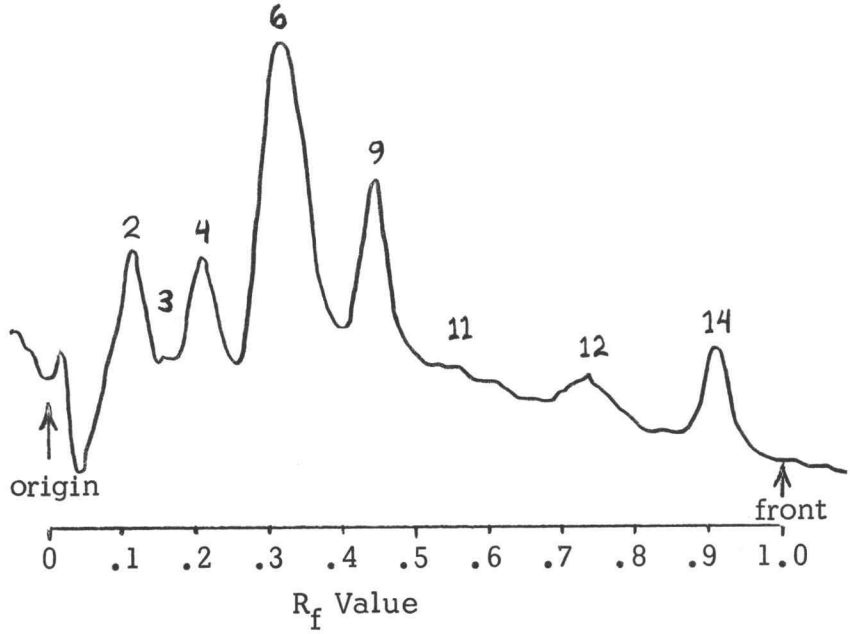
TABLE 19. RELATIVE MOBILITY (R_f) VALUES FOR FEMALE HEMOLYMPH ESTERASE

Age	Band Number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Number of Bands 7-15
1 day	.061	.094	.114	.192	.212	.302		.413		.535	.592	.73		.89		5
2 day		.064	.116	.188		.276		.408	.468	.54	.56	.68		.872		6
3 day		.1		.204	.233	.311			.452		.589	.707		.921		4
4 day		.075	.103	.185	.209	.303		.41		.512	.607	.741		.903		5
5 day		.076	.12	.196		.3		.408		.524	.608	.684		.884		5
6 day		.114		.22		.341			.498	.549	.627	.73		.898		5
7 day		.095	.121	.201		.322		.424	.485	.541		.723		.92	.94	6
14 day	.027	.070		.191		.305			.481	.555		.704		.88	.906	5
1 month	.029	.067		.143		.254			.446		.6			.788		3
2 month	.024	.081	.113	.206		.304				.539	.640	.700		.891		4
3 month		.072	.136	.188		.256		.412	.464		.612			.788		4
4 month	.021	.079		.202		.285			.483		.583	.68		.843	.884	5
5 month	.028	.095	.158	.217		.292		.415		.518		.716		.866	.91	5
6 month	.031	.086		.212		.294			.490			.690		.839	.902	4

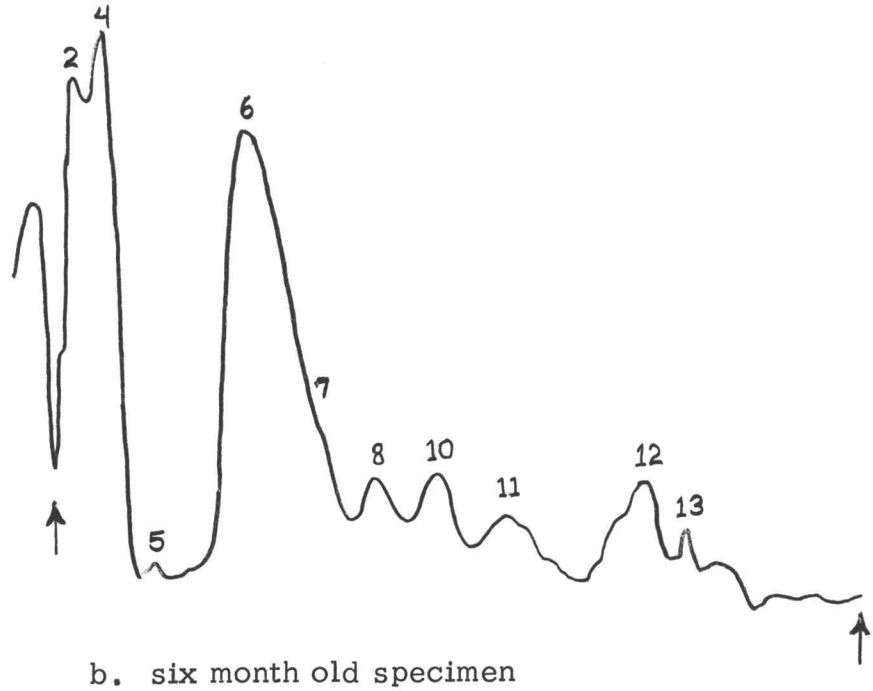
and female pattern, the variation in the two values might be enough to cause them to be placed in different columns. Therefore, because of difficulties in determining the exact point of origin between stacking and separating gel, two "comparable" bands might have widely varying R_f values. Bands 8 and 9 appeared to be fairly distinct and band 8 was often present as a major peak. Band 10 usually was present as a minor peak frequently absent from male patterns. Band 11 turned out to appear more often than band 10 and occasionally as a major band. Band 13 was observed only once, in a one week old male specimen. Bands 12 and 14 were consistent, fairly prominent bands, with band 14 predominating. Band 15, when present, appeared as a double band of 14 and, in some cases, quantitatively exceeded the latter.

Generally, the results for blood esterase patterns of both sexes appear to be characterized by three major slower migrating bands and from three to six (usually four) minor fast migrating bands. There was no correlation between number of minor bands, sex or age. Only the major band 4 showed a tendency to decrease in density with age.

Cook and Forgash (1965) reporting on the esterases in Periplaneta hemolymph, found three heavily staining fractions which agreed with the findings of this study. On the other hand, their total of five hemolymph esterase bands was considerably less than the average total of nine esterase bands found in this study. As indicated in the section on muscle esterases, this may reflect the effect



a. two day old specimen



b. six month old specimen

Figure 39. Densitometric scans of male hemolymph esterase.

TABLE 20. RELATIVE MOBILITY (R_f) VALUES FOR MALE HEMOLYMPH ESTERASE

Age	Band Number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Number of Bands 7-15
1 day		.115		.223	.246	.338			.45		.561	.731		.939		4
2 day		.11	.161	.213		.315			.445		.547	.736		.914		4
3 day	.067	.091	.142	.213	.241	.307			.445		.623	.681		.902		4
4 day		.113	.153	.229		.325				.535	.635	.748		.884	.948	5
5 day	.049	.089	.142	.219		.308	.352		.457		.635			.9	.96	5
6 day		.103		.194		.3		.407		.506		.7		.92	.99	5
7 day		.096	.154	.217		.33					.55	.667	.796	.938		4
14 day	.053	.074		.191		.300		.413		.537	.615	.697		.894	.954	6
1 month		.097		.182		.297		.425		.517	.629	.734		.896		5
2 month		.08	.1	.188		.299		.413		.536	.64	.731		.911		5
3 month	.043	.086		.173		.31	.361	.416		.49	.604	.714		.905		6
4 month	.026	.063		.174		.285			.456		.556	.674		.896	.970	5
5 month	.04	.088		.18		.308		.416				.704		.9	.964	4
6 month		.066		.129	.174	.248	.343	.41		.491	.58	.72	.81			3

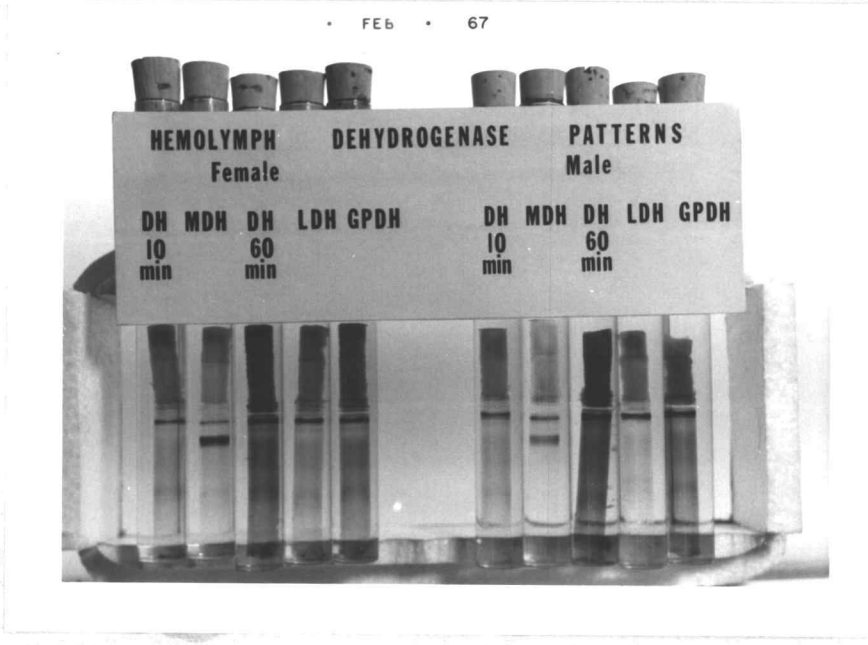


Figure 40. Photograph of specific versus non-specific hemolymph dehydrogenase gels.

of pH in their enzyme development.

Hemolymph esterase patterns with their three main bands signify more stability than was encountered with muscle esterase patterns. However, the variation in minor, fast migrating bands would probably result in patterns too inconsistent to be adequately compared with esterase patterns from other insect hemolymph.

Hemolymph Malic Dehydrogenase Patterns

Muscle MDH zymograms closely resembled those of the hemolymph. Comparison of the non-substrate control with the MDH-substrate gel (Figure 40), revealed only one MDH-specific band (8) with the remainder exhibiting non-specificity (Figures 43a, b and 44a, b). Two main non-specific bands, 3 and 5, varied only slightly in concentration. Band 1, a minor band, appeared only twice during the experiment. The R_f values for bands 2 and 3 of hemolymph closely resembled bands 1 and 2 of muscle patterns and behaved similarly with increasing age. Band 4 usually appeared as a slight peak in the trough between bands 3 and 5 (Figure 44a, b) and band 6 was represented by an inflection point on the leading edge of peak 5. Band 7 was located in the trough between 5 and 8. The predominant and only MDH-specific band 8 in the hemolymph pattern corresponded to the area of greatest activity in muscle MDH patterns. However, only one band was present in hemolymph compared to MDH-specific bands 7,

8 and 9 in muscle. Hemolymph MDH patterns did not possess the heavily staining orange band that was characteristic of all of the muscle dehydrogenases previously described (Figure 41 and 42). They did possess a faintly staining band which possessed approximately the same R_f value but did not have the appearance of the orange band.

Hemolymph MDH patterns showed no excessive variation as a result of aging or because of sexual differences. There was little or no variation in patterns when two specimens of the same age and sex were compared. Hemolymph of females over one month of age lacked band 6, but this was not apparent in the males. However, in males, but not females, increasing age was associated with a decrease in band 5.

Gilbert and Goldberg (1966) found two forms of MDH in the muscle of both sexes and in male hemolymph of Leucophaea, but found only the denser, more rapidly migrating species in female hemolymph. In this study, Periplaneta differed by displaying only one heavily staining MDH band in hemolymph for both sexes. This single band in Periplaneta may be equivalent to the dense, rapidly migrating band in Leucophaea even though the R_f value for the Periplaneta band exceeded that reported in Leucophaea. Because of their similar mobilities, the MDH of Periplaneta hemolymph and muscle may also be equivalent, although the hemolymph band did not display the sub-banding characteristic of the main MDH band in

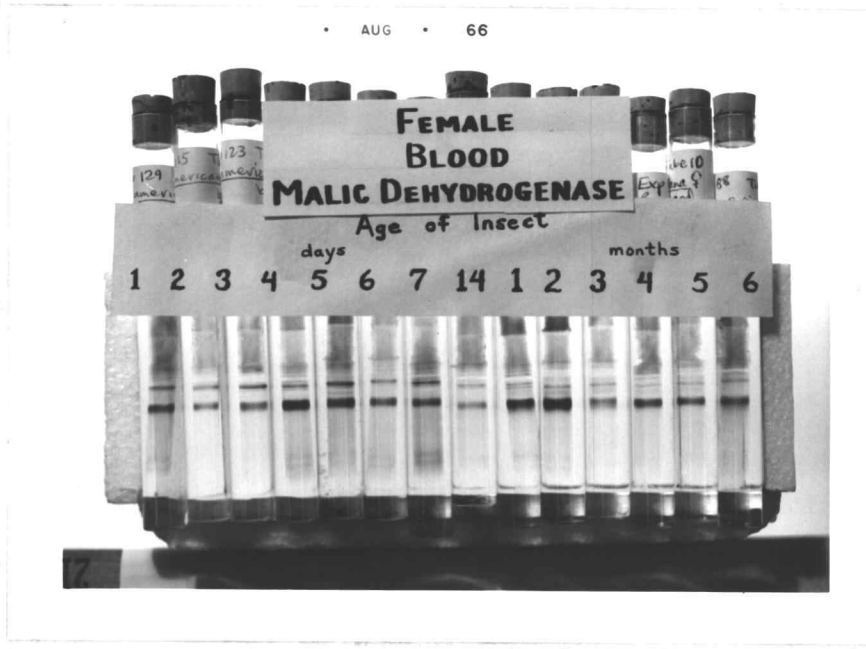


Figure 41. Photograph of female hemolymph malic dehydrogenase gels.

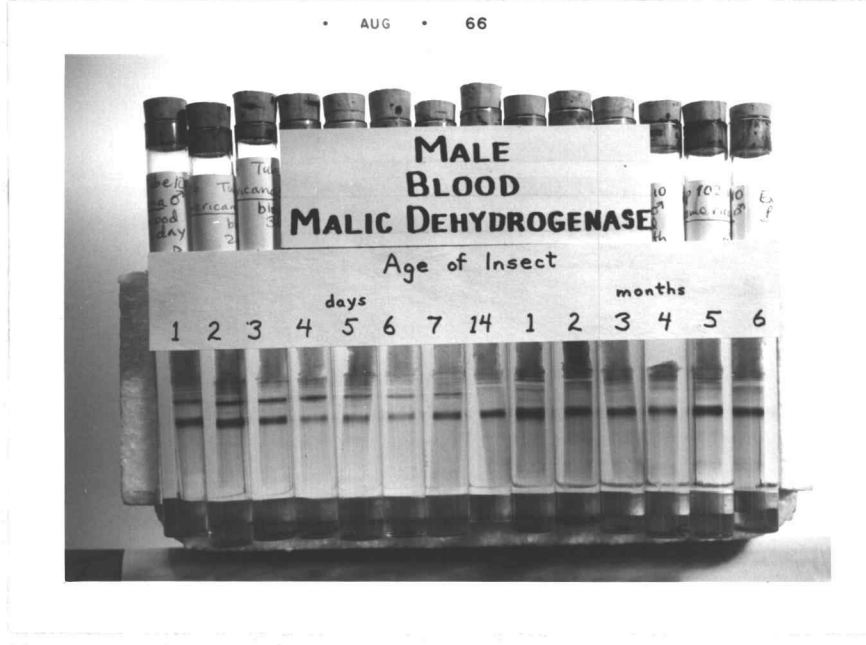
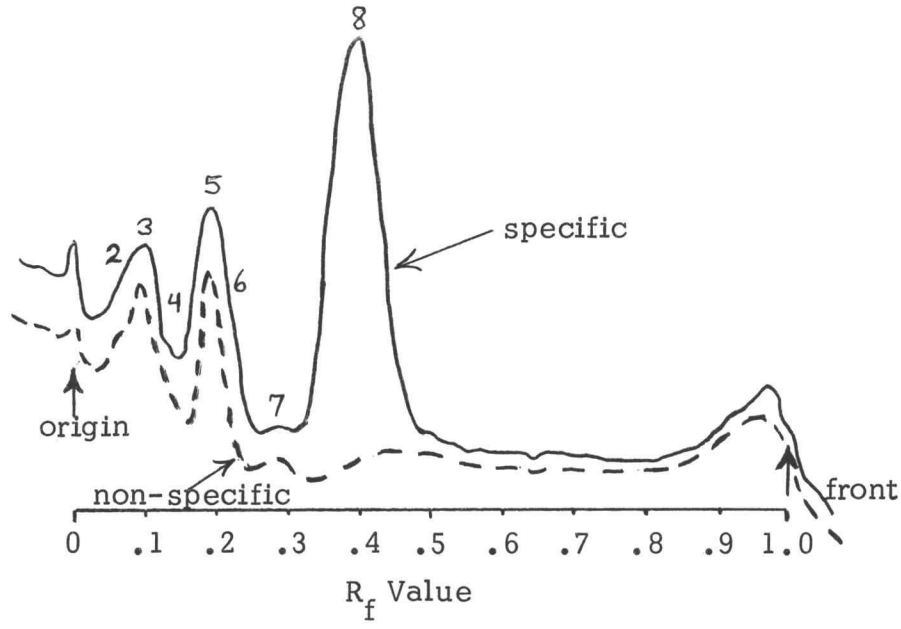
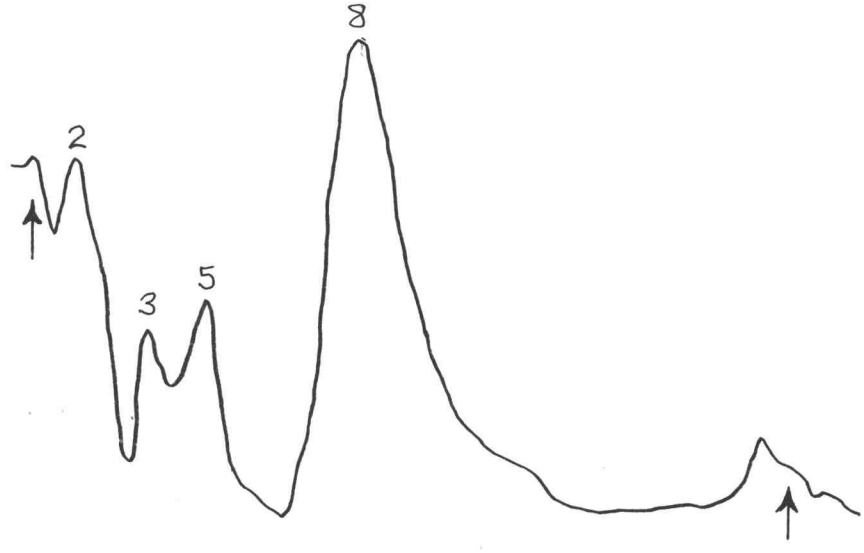


Figure 42. Photograph of male hemolymph malic dehydrogenase gels.



a. two day old specimen

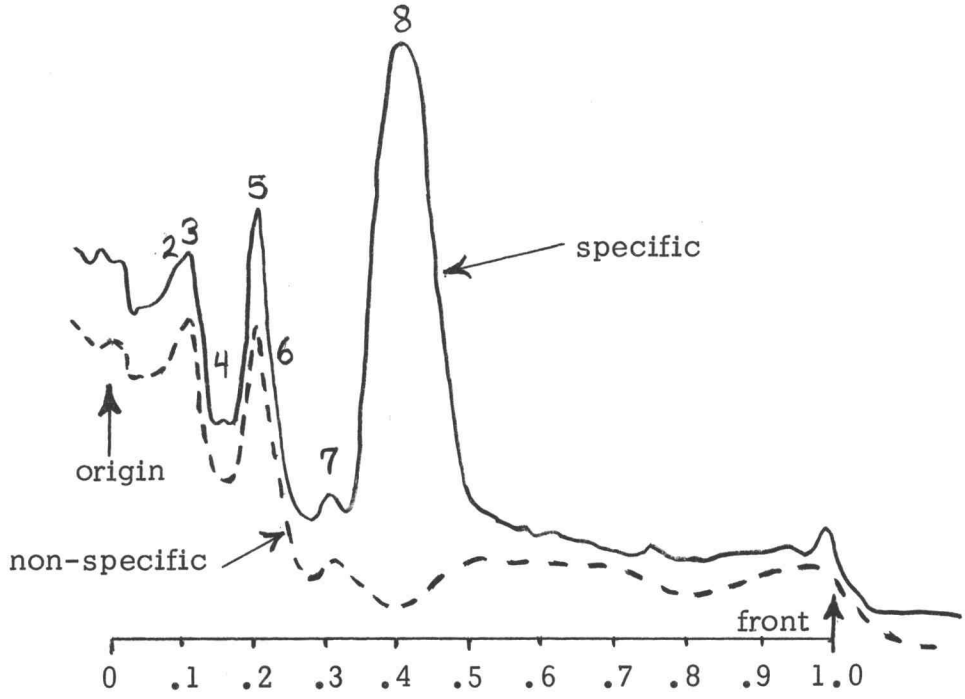


b. six month old specimen

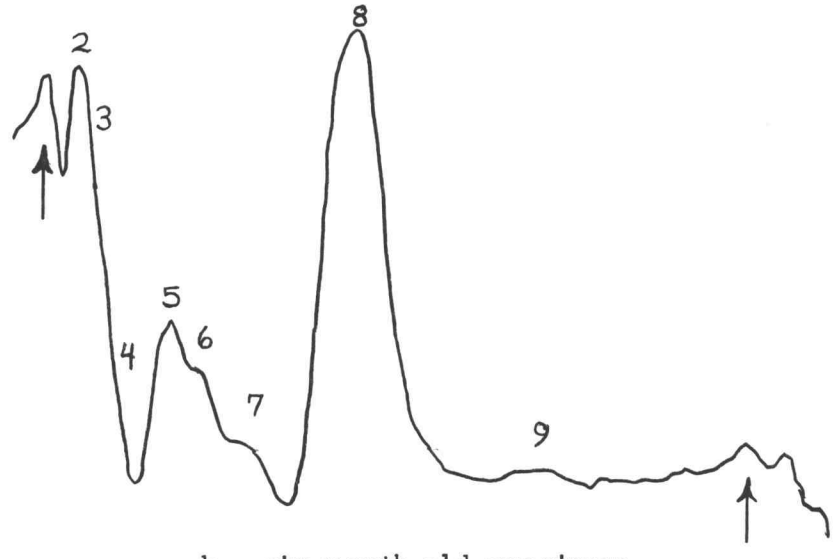
Figure 43. Densitometric scans of female hemolymph malic dehydrogenase.

TABLE 21. RELATIVE MOBILITY (R_f) VALUES FOR FEMALE HEMOLYMPH MALIC DEHYDROGENASE

AGE	Band Number 1	2	3	4	5	6	7	8	9
1 day	.039	.062	.089	.116	.173	.197	.273	.377	
2 day		.061	.102	.134	.195	.224	.285	.403	
3 day		.054	.097	.143	.19	.221	.291	.385	
4 day		.064	.11	.148	.201	.228	.292	.402	
5 day		.069	.119	.146	.219	.25	.313	.415	
6 day		.057	.094	.125	.211	.238	.306	.423	
7 day		.04	.084	.133	.185	.213	.294	.393	
14 day	.04	.06	.096	.156	.212	.24	.308	.424	
1 month		.051	.097	.124	.187	.226	.261	.390	
2 month		.045	.104	.179	.238		.301	.413	.647
3 month		.057	.118	.203	.255		.328	.438	.743
4 month		.046	.092	.139	.220		.282	.405	.71
5 month		.04	.1	.164	.22		.284	.412	.644
6 month		.050	.146		.222		.345	.421	.651



a. two day old specimen



b. six month old specimen

Figure 44. Densitometric scans of male hemolymph malic dehydrogenase.

TABLE 22. RELATIVE MOBILITY (R_f) VALUES FOR MALE HEMOLYMPH MALIC DEHYDROGENASE

Age	Band Number 1	2	3	4	5	6	7	8	9
1 day		.047	.086	.117	.202	.234	.311	.416	
2 day		.085	.105	.153	.206	.234	.302	.407	.75
3 day		.056	.092	.136	.196	.228	.28	.404	
4 day		.068	.1	.14	.192	.219	.287	.41	
5 day	.023	.05	.07	.12	.171	.233	.279	.407	
6 day		.06	.098	.125	.192	.219	.298	.393	
7 day		.064	.105	.139	.192	.226	.301	.402	
14 day		.052	.104	.132	.192	.228	.296	.416	
1 month	.035	.061	.088	.142	.195	.211	.291	.425	.755
2 month		.05	.099	.137	.206	.259	.32	.434	.659
3 month		.048	.116		.208	.264	.304	.432	.676
4 month		.041	.070	.148	.188	.221	.303	.410	
5 month		.055	.111		.194	.229	.301	.439	.672
6 month		.048	.077	.117	.173	.218	.290	.431	.685

Periplaneta muscle.

Hemolymph Lactic Dehydrogenase Patterns

Although hemolymph lactic dehydrogenase patterns exhibited a number of bands, none of these was found to be LDH-specific. The patterns obtained both with and without lactic acid substrate were essentially identical (Figure 40).

A total of 13 non-specific hemolymph LDH bands were found of which only four (2, 5, 6 and 7) are considered main members (Figure 47a). These LDH non-specific bands up to and including band 7 exhibited heavier staining than the corresponding MDH non-specific bands solely because of their longer incubation time. Bands 1, 2, 3, 5 and 6 in hemolymph LDH patterns corresponded with bands 1 through 5 in muscle LDH patterns. Band 7 of hemolymph patterns compared quite closely to band 6 of muscle patterns although the R_f values were not identical. Hemolymph band 4 was usually not resolved on the densitometer but its presence could be determined visually. The location of this band was in a trough between bands 2 and 5. The minimum point of this trough was lower in hemolymph than that found in muscle. Occasionally, a band occurred in position 6a seemingly more prevalent in females. Variation in the pattern became more extensive with the relatively infrequently appearing minor band 9. Bands 10 through 13 were often very difficult to determine since the

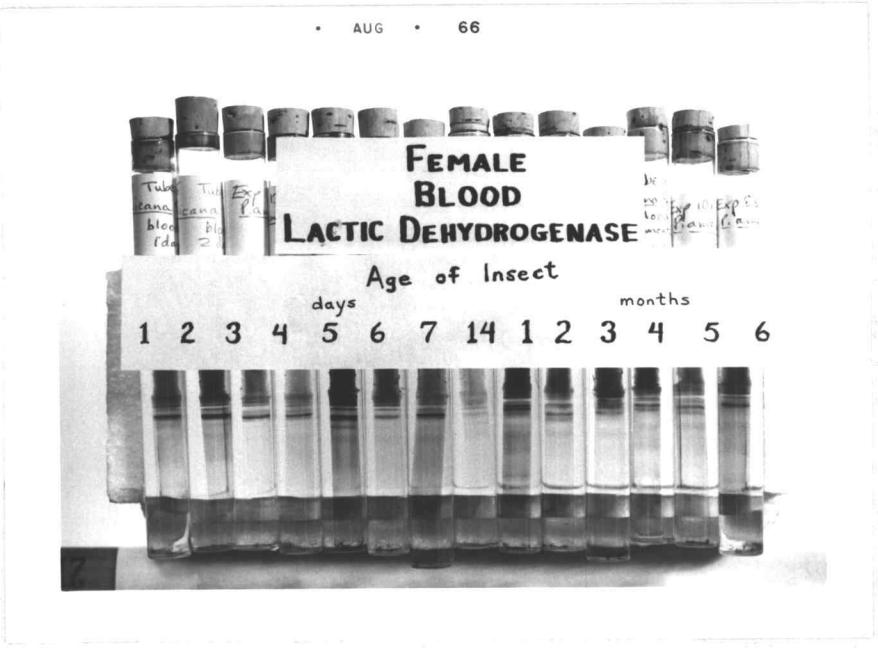


Figure 45. Photograph of female hemolymph lactic dehydrogenase gels.

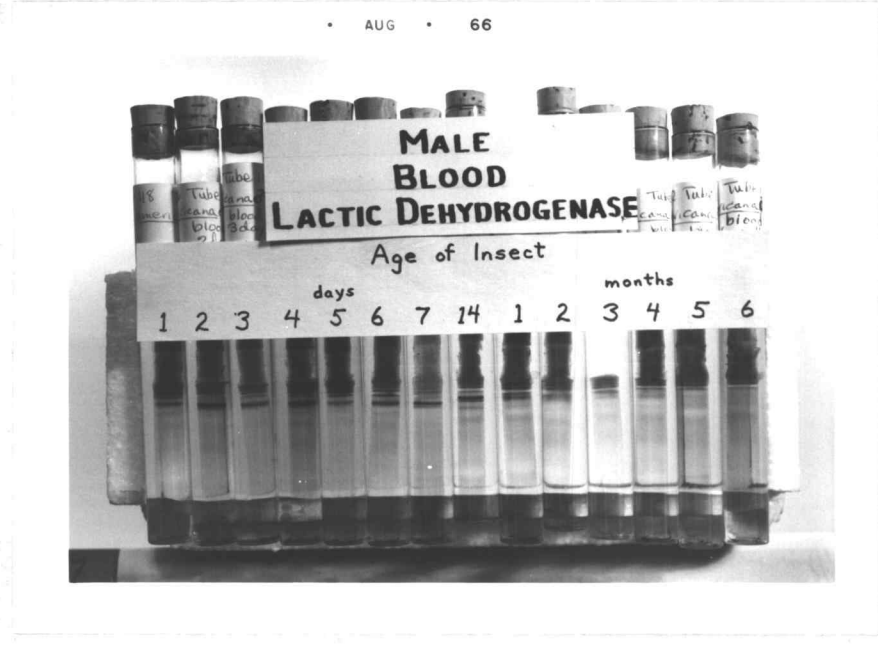
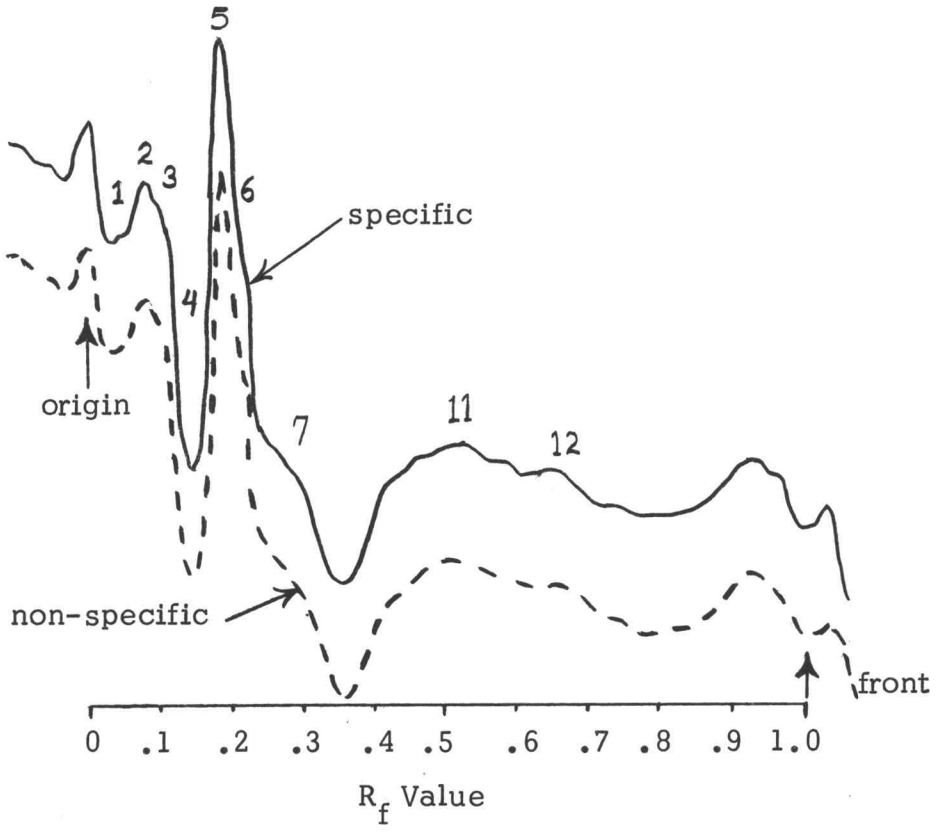
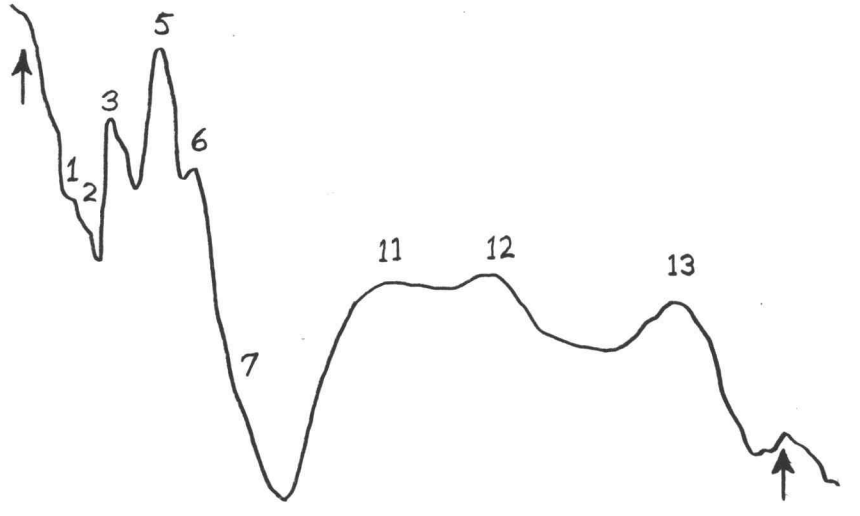


Figure 46. Photograph of male hemolymph lactic dehydrogenase gels.



a. one day old specimen

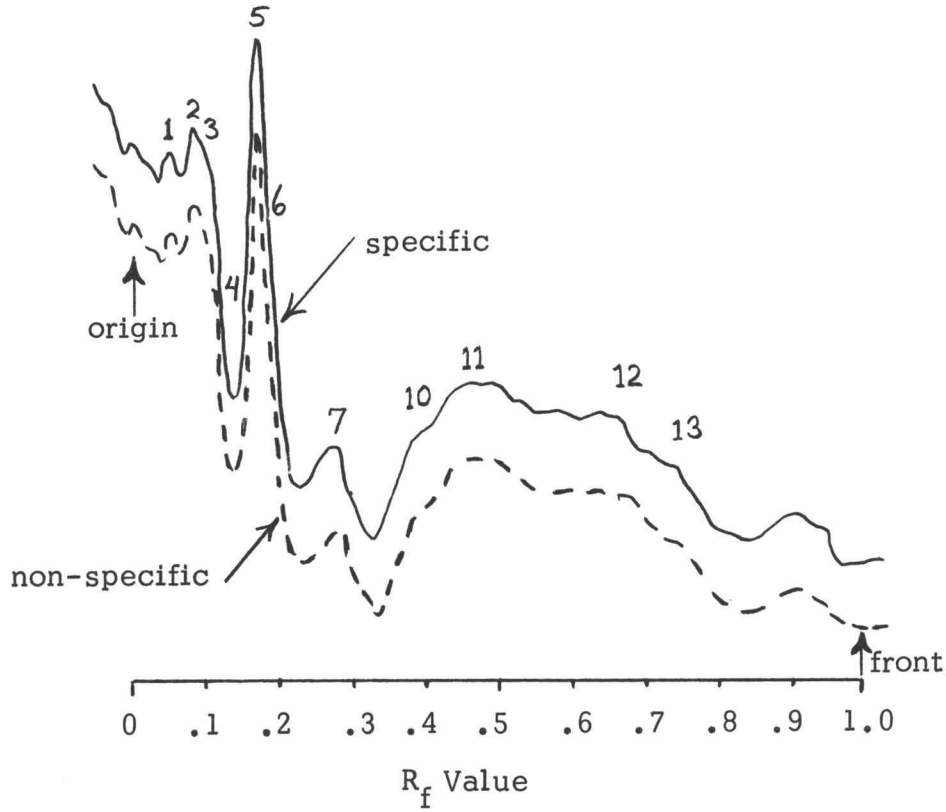


b. four month old specimen

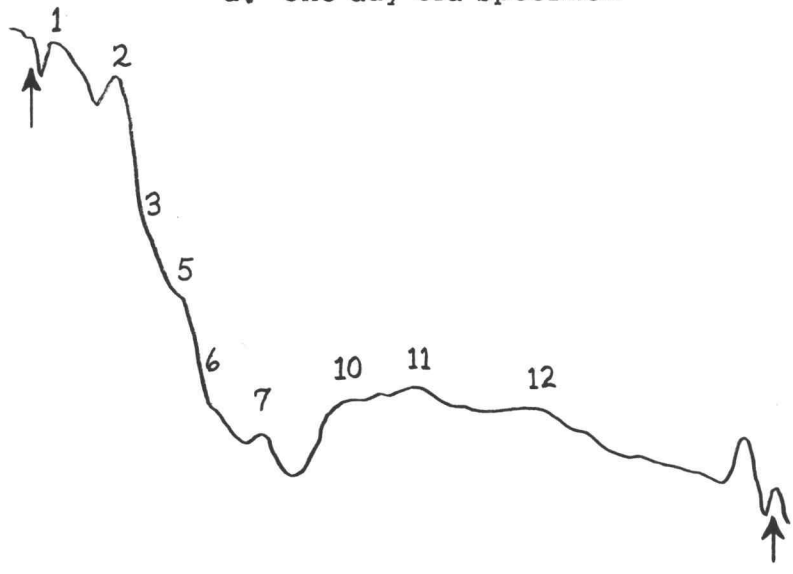
Figure 47. Densitometric scans of female hemolymph lactic dehydrogenase.

TABLE 23. RELATIVE MOBILITY (R_f) VALUES FOR FEMALE HEMOLYMPH LACTIC DEHYDROGENASE

Age	Band Number												
	1	2	3	4	5	6	6a	7	9	10	11	12	13
1 day	.044	.079	.095	.135	.183	.206		.282			.508	.643	
2 day	.028	.072	.096	.132	.184	.212		.272	.328	.392	.436	.64	
3 day	.052	.075	.107	.135	.194	.222		.29		.416	.531	.628	.889
4 day	.052	.1	.128		.18	.212		.284	.32	.424	.516	.644	
5 day	.048	.077	.114	.14	.199	.228		.287		.397	.482	.661	.86
6 day	.042	.062	.094	.119	.168	.189	.217	.259	.297	.381	.454	.63	.7
7 day	.026	.067	.104		.175	.201	.227	.313		.39	.498	.684	.736
14 day	.058	.100		.135	.181	.208	.234	.277		.4	.481	.585	
1 month	.048	.096		.152	.192	.228		.272		.416	.516	.712	
2 month	.042	.102		.158	.23			.283		.43	.511	.665	
3 month	.055	.084	.117	.147	.201	.22		.264	.308	.402	.491	.641	.890
4 month	.056	.108	.126		.175	.216		.286			.476	.595	.836
5 month	.043	.102		.169	.228			.283		.424	.502	.655	
6 month	.049	.089	.137	.171	.213	.251		.3		.41	.498	.65	.722



a. one day old specimen



b. two month old specimen

Figure 48. Densitometric scans of male hemolymph lactic dehydrogenase.

TABLE 24. RELATIVE MOBILITY (R_f) VALUES FOR MALE HEMOLYMPH LACTIC DEHYDROGENASE

Age	Band Number												
	1	2	3	4	5	6	6a	7	9	10	11	12	13
1 day	.052	.083	.099	.135	.171	.198		.278		.39	.469	.67	.742
2 day	.062	.096		.146	.204	.235		.3			.508	.639	
3 day	.069	.089		.134	.19	.233	.275	.308			.498	.62	
4 day	.043	.071	.098	.145	.188	.224		.282		.404	.518	.635	.812
5 day	.058	.111		.157	.218	.246		.317		.441	.547	.687	
6 day	.041	.074	.111	.14	.188	.218		.288		.395	.59	.657	.835
7 day	.039	.081	.112	.139	.181	.216		.316		.404	.5	.646	.843
14 day	.048	.099	.131	.166	.206	.237		.316		.49	.53	.66	.858
1 month	.046	.1	.15		.196	.211		.283		.429	.512	.696	
2 month	.036	.116	.16		.204	.252		.324		.432	.532	.704	
3 month	.054	.074	.124		.214			.284		.413	.527	.68	
4 month	.031	.077	.104		.146	.177		.304		.406	.488	.638	.792
5 month	.041	.067	.111		.197	.245				.445	.526	.678	
6 month	.047	.086			.141	.173				.427	.518	.686	

bands were situated in an area of relatively high background staining, and the resulting variation in R_f values for these bands may be attributable to this. A noteworthy characteristic in hemolymph patterns was the absence of the orange staining band which was so characteristic of muscle LDH patterns (Figures 45 and 46).

Increasing age did not materially alter hemolymph LDH patterns and sexual differences were slight. Patterns of specimens less than one month were similar except for the occasional absence of band 3 in males and the occasional presence of band 9 in females. Older females tended to lose band 3, but this was not at all consistent. Band 4 was retained in older females but lost in older males. Bands 5 and 6 were retained in older specimens of both sexes, although band 5 of the male was greatly reduced in magnitude. Band 9 did not appear in male specimens of any age. There did not appear to be any age or sex associated variation in bands 7 through 13.

The conclusions concerning the usefulness of the LDH enzyme in hemolymph will be summarized in the section covering hemolymph GPDH patterns.

Hemolymph α-glycerophosphate Dehydrogenase Patterns

As with LDH, there were no GPDH-specific bands in hemolymph. Consequently, the gels incubated with α-glycerophosphate substrate were identical to the gels in which the substrate was omitted (Figure 40).

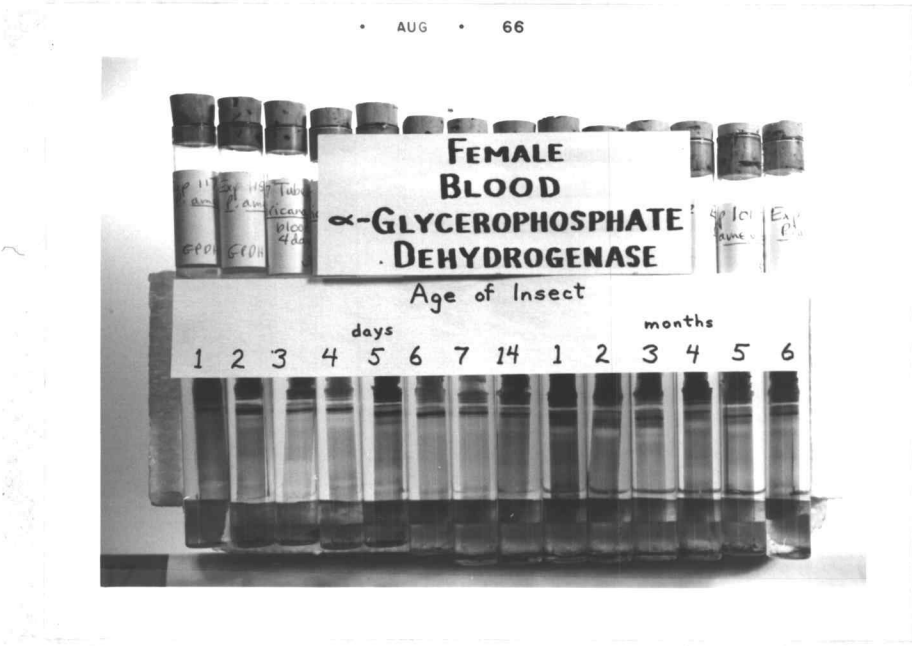


Figure 49. Photograph of female hemolymph α-glycerophosphate dehydrogenase gels.

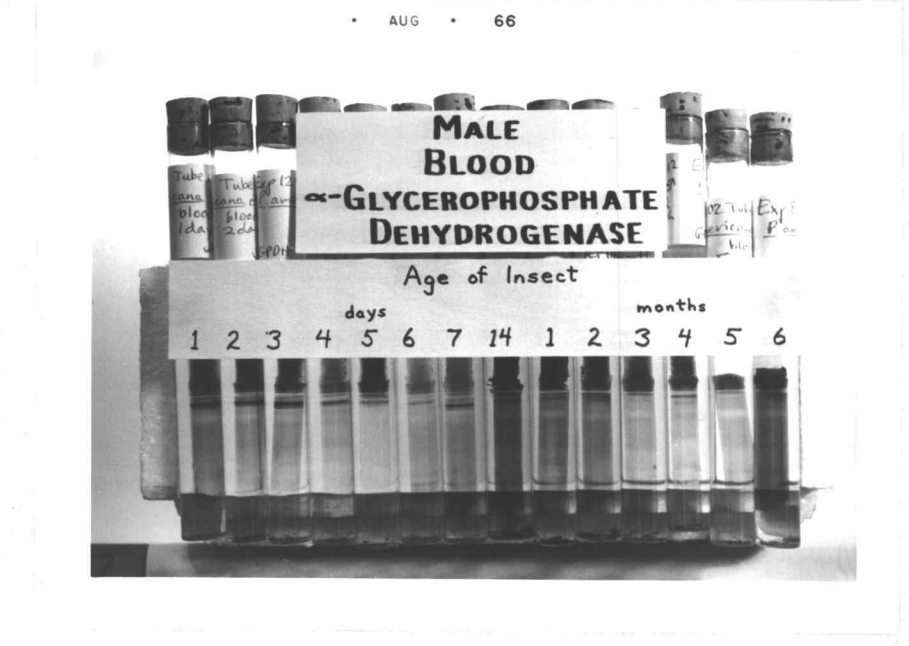
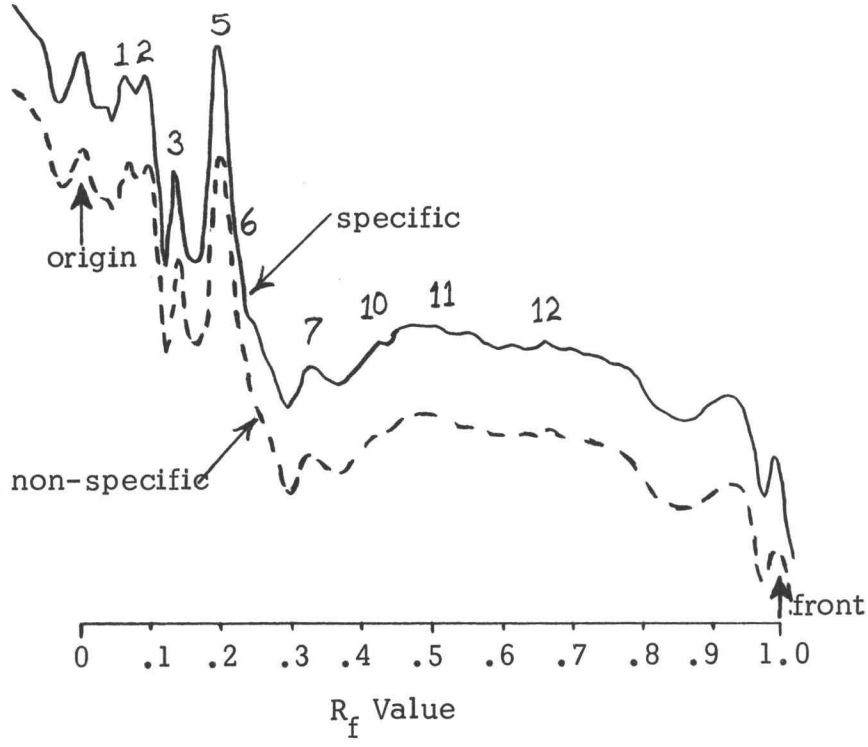
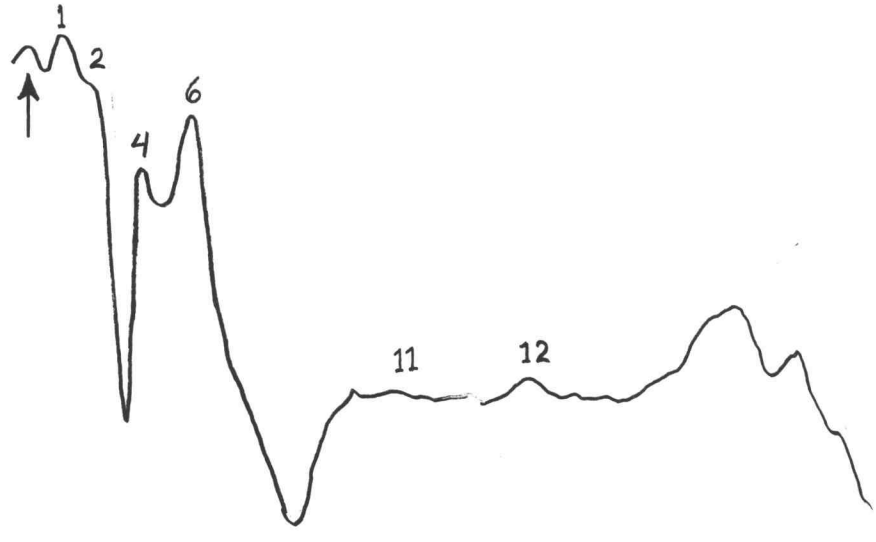


Figure 50. Photograph of male hemolymph α-glycerophosphate dehydrogenase gels.



a. one day old specimen

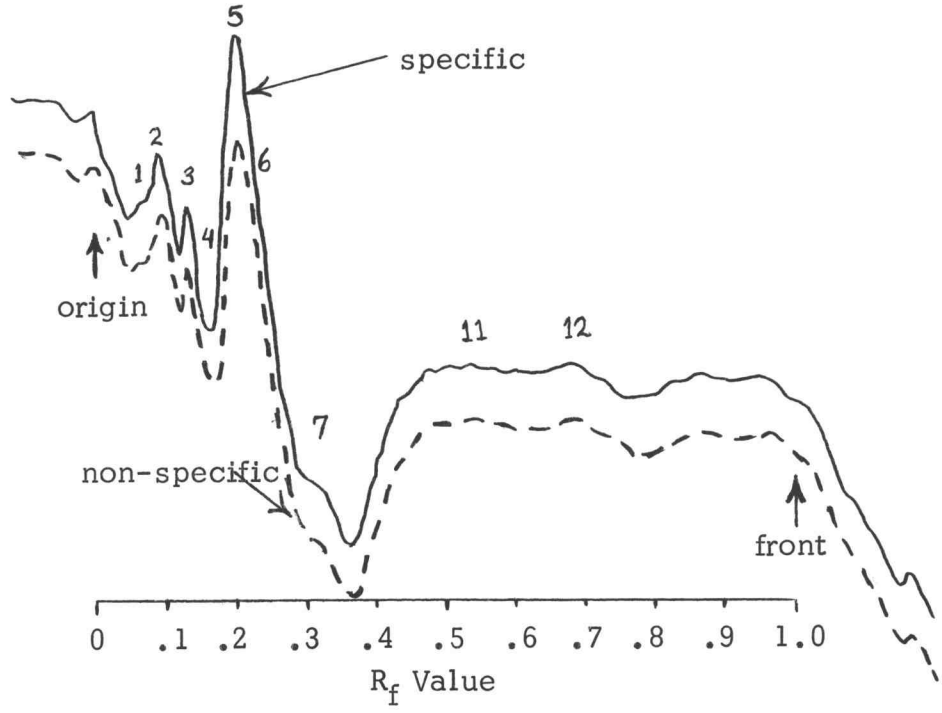


b. six month old specimen

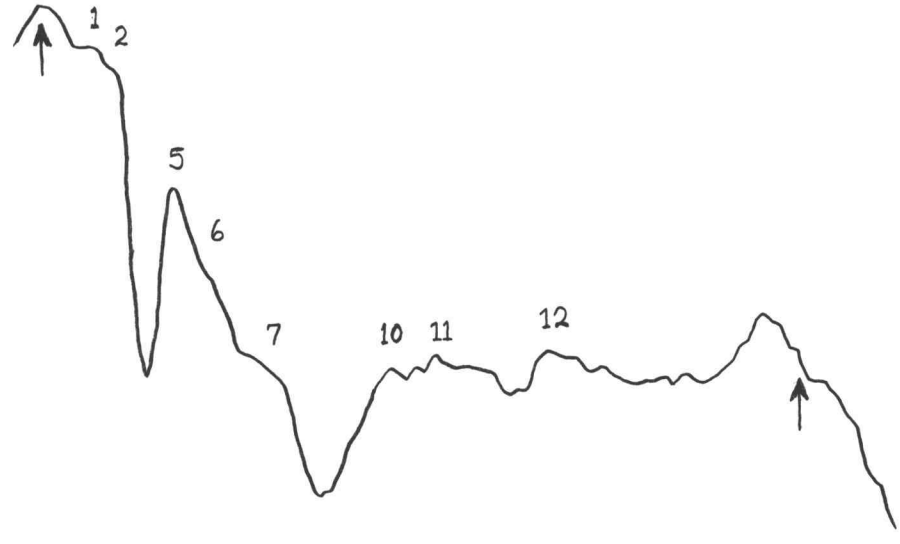
Figure 51. Densitometric scans of female hemolymph α-glycerophosphate dehydrogenase.

TABLE 25. RELATIVE MOBILITY (R_f) VALUES FOR FEMALE HEMOLYMPH α-GLYCEROPHOSPHATE DEHYDROGENASE

Age	Band Number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
1 day	.06	.088	.128		.188	.212	.252	.312		.408	.487	.644	
2 day	.068	.104	.133		.197	.225		.289			.461	.647	.895
3 day	.063	.096	.115		.178	.207		.27	.296	.378	.437	.648	.789
4 day	.049	.081	.102	.13	.179	.212		.285	.309	.415	.485	.684	
5 day	.034	.072	.121		.197	.224		.292		.402	.462	.66	.875
6 day	.052	.089	.104	.145	.193	.222	.259	.313		.408	.481	.74	
7 day	.056	.070	.112	.157	.194	.224		.309		.416	.518	.636	
14 day	.064	.096	.151	.179	.215	.247		.303		.434	.538	.717	
1 month	.052	.144	.168		.204	.244		.284		.428	.532	.785	
2 month	.056	.108	.144	.176	.236	.268					.488	.680	.712
3 month	.054	.13		.198	.253			.326		.433	.548	.728	
4 month	.06	.087	.124	.169	.218			.256			.425	.651	
5 month	.044	.1		.164	.22			.28			.48	.706	.76
6 month	.044	.08		.144	.216			.288			.484	.676	



a. one day old specimen



b. six month old specimen

Figure 52. Densitometric scans of male hemolymph α-glycerophosphate dehydrogenase.

TABLE 26. RELATIVE MOBILITY (R_f) VALUES FOR MALE HEMOLYMPH α-GLYCEROPHOSPHATE DEHYDROGENASE

Age	Band Number 1	2	3	4	5	6	7	8	9	10	11	12	13
1 day	.042	.072	.11	.131	.186	.22	.249	.295			.598	.683	
2 day	.038	.058	.092	.135	.185	.215		.277		.392	.512	.673	
3 day	.041	.065	.085	.122	.191	.224		.285		.418	.512	.659	
4 day	.033	.074	.112		.186	.219		.29		.413	.517	.661	
5 day	.036	.096	.124		.204	.246		.308		.432	.512	.712	
6 day	.047	.086	.11		.189	.216	.259	.298		.431	.502	.776	
7 day	.052	.091	.121	.149	.194	.226		.323		.411	.516		.879
14 day	.078	.119	.152		.185	.215	.237	.281			.437	.555	.793
1 month	.047	.075	.142		.185	.209		.28		.441	.531	.685	.866
2 month	.062	.099	.154		.209			.275			.473	.641	
3 month	.054	.083	.117		.221			.275		.429	.55	.712	
4 month	.033	.081	.109		.205			.286		.438	.514	.714	
5 month	.035	.065	.126		.211			.272		.448	.548	.682	.862
6 month	.066	.097			.181	.224		.317		.46	.521	.676	

The non-specific bands of LDH and GPDH were therefore identical.

It is uncertain why repeated experimentation failed to produce any LDH- or GPDH-specific patterns in hemolymph. In future studies, it would be necessary to identify the non-specific tetrazolium binding fractions which are so prevalent in hemolymph as well as muscle. Until that time, it is apparent that neither hemolymph LDH or GPDH can be used as biochemical indicators of relationship.

SUMMARY

In this study, the thoracic muscle, appendage muscle and hemolymph of Periplaneta americana were examined for isozymic forms of esterase, malic dehydrogenase, lactic dehydrogenase and α-glycerophosphate dehydrogenase in specimens aged one through seven days, two weeks, and one through six months. It was discovered that the correct protein concentration was a critical factor in facilitating photopolymerization of the sample gel. An effort was made to keep this level below approximately 4 mg/ml.

Esterase patterns in muscle were characterized by a total complement of sixteen bands with each pattern averaging eight to thirteen bands. Although the six major bands displayed reasonable stability, a considerable variation was exhibited by the minor bands. One band of R_f approximating .61 was found to be more prominent in the patterns of male thoracic muscle and the appendage muscle of both sexes than in those of female thoracic muscle. An obvious effect of aging was the increased resolution of the fastest migrating esterase band in the muscle of most specimens older than seven days. In addition, a buffer of pH 5.6 in the esterase incubation mixture was found to be essential for the development of the highest complement of bands. Because the muscle esterases display a considerable degree of variability, they may be of questionable value in the study of biochemical

systematics.

Dehydrogenases differed from the esterases in that they were characterized by non-specific banding as a result of eliminating the substrate from the incubation mixture. This phenomenon was true for all three tissues tested and non-specific staining accounted for the majority of the activity in the gel.

The pattern exhibited by muscle MDH was characterized by a main specific band complex consisting of three bands in the vicinity of R_f .39 and two minor slower migrating bands. The minor bands were more prominent in male muscle, but aging caused the minor bands to become fainter and the main band complex to coalesce. The lack of significant variation between specimens of similar age and sex would qualify this enzyme as a possible indicator for further studies in systematics.

Lactic dehydrogenase muscle patterns of both sexes exhibited only one LDH-specific band with an R_f value of approximately .5 which may correspond to the .34 R_f value of the most rapidly migrating LDH band in the tissues of Leucophaea maderae. The Periplaneta LDH patterns were also characterized by a clear area on the gel possibly due to an iron binding protein which interferes with the staining mechanism. Because of stability of the main LDH band in Periplaneta muscle throughout the experimentation, this enzyme may have value in biochemical systematics.

Muscle patterns of α-glycerophosphate dehydrogenase exhibited one main band which displayed some variation with respect to R_f value and density. In female thoracic muscle tissue this main band is usually not present, while in the other muscle of both sexes it was represented by a heavy staining single (R_f approximately .42) or double band. This R_f value compares closely to the .48 value for Leucophaea muscle GPDH. The variation in this main band, however, would make comparisons with other insect muscle GPDH patterns difficult.

The three major hemolymph esterase bands exhibited in Periplaneta are comparable in position and relative density to three main esterases reported earlier for Periplaneta, but this study also disclosed from three to six more rapidly migrating minor bands. One of these three major bands decreased in density with increasing age. The degree of variation exhibited by the minor bands may prevent meaningful comparisons with patterns from hemolymph of other insect species.

One main malic dehydrogenase specific band was recovered from the hemolymph of both sexes and its position was comparable to that of the MDH-specific three band complex in muscle. This main hemolymph band was also similar to R_f to the main MDH band found in the hemolymph of both sexes of Leucophaea. There was little, if any, change in this band in Periplaneta with increasing age and it would be a possible candidate for future biochemical systematic comparisons.

All of the enzymes tested failed to exhibit any great metachemogenetic effect in the three tissues studied in Periplaneta. With the

exception of the esterases, the remaining dehydrogenase patterns displayed great stability when several specimens of the same or different ages were compared. In most cases, the patterns of a particular enzyme also appeared to be quite similar for both sexes of the same age and did not display any drastic changes with increasing age. Except for GPDH in female thoracic muscle and LDH and GPDH in hemolymph, the three dehydrogenases could be profitably employed as systematic tools. Esterases could be better utilized in biochemical systematic studies by the addition of selective enzyme inhibitors to eliminate much of the activity and source of variation due to non-specific esterase staining. Further investigation is needed to elucidate the possible cause for the variable appearance of the GPDH-specific band in muscle and to also uncover the mechanism of non-specific band activity of these dehydrogenases.

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