8-1978

Lactate dehydrogenase isozyme patterns from five species of snakes : comparison of rattlesnake vibratory muscle with body epaxial muscle

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LACTATE DEHYDROGENASE ISOZYME PATTERNS FROM FIVE SPECIES OF SNAKES: COMPARISON OF RATTLESNAKE VIBRATORY MUSCLE WITH BODY EPAXIAL MUSCLE

BY

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A THESIS

SUBMITTED TO THE GRADUATE FACULTY

OF THE UNIVERSITY OF RICHMOND

IN CANDIDACY

FOR THE DEGREE OF

MASTER OF SCIENCE

IN BIOLOGY

AUGUST 1978

LACTATE DEHYDROGENASE ISOZYME PATTERNS

FROM FIVE SPECIES OF SNAKES: COMPARISON OF RATTLESNAKE

VIBRATORY MUSCLE WITH BODY EPAXIAL MUSCLE

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ACKNOWLEDGEMENTS

The author is grateful to Dr. Gerald C. Schaefer, his major professor, for his guidance and assistance throughout the research and writing of this thesis. Futhermore I should like to express my appreciation to my committee members, Dr. Francis B. Leftwich and Dr. William S. Woolcott, for their comments and suggestions that have made this a better thesis. I should like to thank Dr. David W. Towle for his invaluable advice on laboratory procedures and Mr. Timothy J. Dietrick (fellow graduate student) for helping pioneer the initial experiment. Also I should like to thank Dr. J. Van Bowen (Department of Mathematics, University of Richmond) for his help with computer programing for the processing and statistical analyses of this study.

I am indebted to Dr. Francis B. Leftwich, who has been both my advisor and friend during the trying periods of my seven years at the University of Richmond. Finally I wish to thank the entire faculty of the Biology Department and the University of Richmond as a whole, which has provided the academic and social environment that has enriched my education and prepared me for the future.

ABSTRACT

Lactate dehydrogenase (LDH) isozyme patterns of seven tissues in each of five species of snakes are described from their polyacrylamide gel electrophoretic characteristics. The tissues examined included vibratory tail muscle, non-vibratory tail muscle, body epaxial muscle, heart muscle, whole blood, liver and kidney. The species of snakes included Crotalus adamanteus, C. h. horridus, Agkistrodon piscivorus and Sistrurus miliarius barbouri from the family Crotalidae and Natrix taxispilota from the family Colubridae. These snakes show a decreasing continuum of tail vibratory activity, respectively, to N. taxispilota, which has a non-vibratory tail muscle.

Crotalus adamanteus, C. h. horridus and A. piscivorus have similar LDH isozyme patterns. They represent a case of restricted subunit association and have only four isozymes rather than five which are characteristic of the majority of vertebrates. The missing heteropolymer was determined to be LDH-4 (A_3B_1). This is the first time that the "four-isozyme" system has been reported in Crotalid snakes. Sistrurus m. barbouri and N. taxispilota show non-restricted subunit association and have the five-isozyme LDH pattern.

Muscles which contract rhythmically tend to show LDH patterns like those in heart muscle, therefore it was

anticipated that rattlesnake vibratory muscle would exhibit a similar pattern. However, this was not the case as vibratory muscle LDH patterns resembled non-rhythmical body epaxial muscle patterns and not those of heart muscle. Vibratory muscle and body epaxial muscle differed in heteropolymer bands LDH-2 (A_1B_3) and LDH-3 (A_2B_2), which were wider in vibratory muscle than in body epaxial muscle. There were positive correlations between muscle vibratory activity and increases in heteropolymer band width and intensity.

Vibratory muscle LDH patterns were found to be like the white skeletal muscle LDH patterns in most vertebrates and similar to liver LDH isozymes. Vibratory muscle and liver have LDH characteristic of anaerobic respiration, however both function in an aerobic environment. Vibratory muscle LDH could represent an alternate energy route during prolonged vibration when oxygen may not be readily available.

INTRODUCTION

The purpose of this study was to determine the lactate dehydrogenase(LDH) isozyme patterns of rattlesnake vibratory muscle and compare them with those of heart and epaxial muscle of rattlesnakes. Snakes other than rattlesnakes were examined to give a comparison between vibratory and non-vibratory tail muscles.

Lactate dehydrogenase catalyzes the last step in glycolysis; i.e. the reduction of pyruvate to lactate (Lehninger, 1975). The LDH isozymes are tetrameric molecules composed of four subunits from two parent molecules. In the past, parent molecules have been designated as M (muscle) and H (heart) for the organs in which they occur predominantly (Dawson, et al., 1964). Current researchers (Everse and Kaplan, 1975; Markert, et al., 1975; Vesell, 1975) replace M and H designations with A and B, respectively. The latter format will be followed in the present study. The two parent molecules A and B are coded by two separate structural genes whose monomeric products combine to form five isozymes. In most vertebrates, these five possible tetramer combinations are: B_4 (LDH-1); A_1B_3 (LDH-2); A_2B_2 (LDH-3); A_3B_1 (LDH-4) and A_4 (LDH-5) (Appella, et al., 1961; Cahn, et al., 1962; Markert, 1962, 1963a, 1963b; Markert, et al., 1975).

Each of the multiple forms of LDH appears to have different functions. They are involved in different modes of metabolism and are specifically characteristic of specific tissues. The LDH present in muscles is characteristic of their physiological function. Muscles which contract tonically or rhythmically tend to have LDH patterns like the heart (B type), whereas muscles which contract more abruptly have LDH patterns consisting primarily of A type molecules (Dawson, et al., 1964).

In the present study, five species of snakes which demonstrate a decreasing continuum of tail vibratory behavior were used to determine muscle LDH patterns. The largest of North American rattlesnakes, Crotalus adamanteus (eastern diamondback rattlesnake), displays a high degree of vibratory activity. A physically smaller species, Crotalus horridus horridus (timber rattlesnake), can be juxtaposed to C. adamanteus in vibratory behavior. A third species, Sistrurus miliarius barbouri (Florida or dusky pigmy rattlesnake) is a dwarf rattlesnake. Although it possesses a rattle, the vibratory muscles are not as well developed as those in Crotalus and it can be considered a primitive rattlesnake (Klauber, 1956). A fourth species, Agkistrodon piscivorus piscivorus and Agkistrodon piscivorus conanti (cottonmouth), is a member of the family Crotalidae, the pitvipers, which

includes Crotalus and Sistrurus, but is a tail vibrator without a rattle. The last species, Natrix taxispilota (brown watersnake), is a non-vibrating member of the family Colubridae (Schaefer, 1978, pers. comm.).

Rattlesnake vibratory muscle eventually may be shown to be the fastest contracting vertebrate muscle (Martin and Bagby, 1973). Because vibratory muscles can contract with a very high frequency over a long period of time it is considered a very highly specialized muscle (Martin and Bagby, 1972, 1973). For seventeen species of rattlesnakes the average rattling frequency is 48 cycles per second (Klauber, 1956) and it has been proposed that frequencies up to 100 cycles per second are possible (Chadwick and Rahn, 1954). Crotalus atrox (western diamondback rattlesnake) has been observed rattling for three hours without stopping and to show little fatigue (Martin and Bagby, 1972). Schaefer (1978, pers. comm.) has, on two separate occasions, observed C. adamanteus rattling continuously for four hours with no signs of fatigue.

Forbes (1967) and Kerins (1969), using tissue oxygen consumption (Q_{O_2}), succinic dehydrogenase activity and cytochrome oxidase activity, have concluded that rattlesnake vibratory muscles are specialized for high respiratory activity. The metabolic specialization is supported by morphological evidence for a high rate of activity.

Vibratory muscle has more glycogen granules and mitochondria than epaxial muscle (Pastore, 1967), which indicates that vibratory muscle has a larger reserve energy supply and a higher capacity for respiration than does ordinary skeletal muscle.

Heart muscle and rattlesnake vibratory muscle have several common properties. Heart muscle is very red and high in enzymes of aerobic metabolism (Prosser and Brown, 1971). The vibratory muscle is also red with high enzyme levels and Q_{O2} (Forbes, 1967; Kerins, 1969), as in heart muscle. Both heart and vibratory muscles contract rhythmically and are capable of contracting for long periods without fatigue. These relationships help lend credence to the prediction that rattlesnake heart and vibratory muscle may have similar LDH patterns.

MATERIALS AND METHODS

Five species of snakes were used in this study of LDH tissue patterns (Table 1). Nine *C. adamanteus* and one *A. p. conanti* were collected at Cherokee Plantation in Leon County, Florida during March, 1977. Four *S. m. barbouri* were obtained commercially from Ray Singleton, Tampa, Florida during April, 1977. Four of the *C. h. horridus* were collected in Wyoming County, Pennsylvania during June, 1977 and one in Highland County, Virginia during May, 1976. The remaining *A. p. piscivorus* (three) were collected in Virginia Beach near Creeds at the Trojan Station, Virginia Commission of Game and Inland Fisheries, Virginia during April, 1976. Six specimens of *N. taxispilota* were collected in Baileys Creek, Prince George County, Virginia during July, 1977.

Each snake was decapitated and blood collected in a beaker as it was pumped out of the severed dorsal aorta. The animals were skinned and dissected tissues placed in beakers on ice. Vibratory muscle was dissected from the three species of rattlesnakes and tail muscle was removed below the cloacal aperture to the tip of the tail from *A. piscivorus* and *N. taxispilota*. Body epaxial muscle was dissected from midway between the cloacal aperture and the head from all snakes. Midsections of the kidney and liver

and the whole heart were also removed.

Tissues from freshly killed animals were washed in ice cold 0.05 M Tris-glycine buffer (pH 9.2), minced and then homogenized in four volumes of the buffer with a glass on glass homogenizer attached to a power stirrer. The homogenizer was placed in an ice bath to help prevent protein deterioration. Homogenates were centrifuged at 5 C and 100,000 g for 15 minutes. Lipoproteins were removed from the supernatant by filtering it through buffer-moistened glass wool. An electrophoretic marker of 0.2 ml 0.05 % Bromphenol blue in 20 % sucrose was added per ml of filtrate. Filtrate samples of 10 ul were applied to polyacrylamide gel surfaces using micropipets (Towle, 1977, pers. comm.). Remaining samples were then frozen for experimental rerun purposes. Frozen samples were stored for only two weeks. Freezing for longer periods results in decreased LDH isozyme activity and may cause LDH isozyme band splitting (Gordan, 1969).

Polyacrylamide gels were prepared using the method developed by Hjerten (1962). The synthetic polymer, polyacrylamide, can be prepared to give an effective median pore radius of 0.5 to 3 nm (Fawcett and Morris, 1966) by adjusting the total acrylamide concentration (acrylamide plus Bis), designated % T, and the concentration of cross-

linking agent (Bis), designated %C (Hjerten, 1962; Fawcett and Morris, 1966). Thus a 16 %T x 1.5 %C gel was prepared from a solution containing 15.76 g of acrylamide and 0.24 g of Bis per 100 ml of distilled water.

The working gel solution involves use of three stock solutions: (A) 15.76 g of acrylamide; 0.24 g of N,N'-Methylene-bis-Acrylamide; (B) 48 ml of 1N HCl; 36.3 g of Tris(Hydroxymethyl)Aminomethane; 0.46 ml of N,N,N',N',-tetramethylethylenediamine and; (C) 0.14 g of ammonium persulfate. Stock solutions were made in 100 ml amounts with distilled water and then deaerated under vacuum to remove atmospheric oxygen. Oxygen is the most important polymerization inhibitor (Chrambach and Rodbard, 1971) and needs to be removed to insure consistant polymerization.

The working gel solution was prepared using 2 parts A, 1 part B, 4 parts C and 1 part distilled water. The exact amount of solution to be used can be prepared by determining the total volume necessary to fill the gel tubes (2 ml per tube) and divide that volume by 8. The result is a volume equal to 1 part of the gel working solution. The working solution can be kept on ice to retard polymerization, but if allowed to warm up, ammonium persulfate will achieve polymerization in 15 to 20 minutes (Chrambach and Rodbard, 1971). Bio-Rad gel tubes were filled to 8 cm with gel

working solution and then topped with 5 mm of distilled water to produce a flat interface.

After polymerization, the tubes were placed in electrophoretic gel chambers. The upper chamber was filled with 500 ml of 0.025 M Tris-glycine (pH 9.5) top buffer and the lower chamber with 650 ml of 0.025 M Tris-HCl (pH 8.2) bottom buffer. Buffer temperature was approximately 8 to 10 C at the start of the experiment. Air bubbles were removed from inside and below the gel tubes with pasteur pipets. For each tissue, 10 ul samples were applied to four tubes between the gel surface and top buffer with micropipets. Gel chambers were connected to a power supply and the current regulated at 25 mA per chamber. The average run time per chamber was two hours.

Gels were removed from tubes with a 20 guage 6 inch hypodermic needle by injecting distilled water between the gel and the inner surface of the tube. Sterile gloves or 3 x 5 cards were used to handle the exposed gels. After removal, gels were placed in petri dishes and stained for 20 minutes in an oven at 37 C. The LDH activity stain contained 8.0 ml of 0.5 M DL-Lactic acid, 6.0 ml of 0.001 M nicotinamide adenine dinucleotide (NAD), 4.0 ml of 0.1 M NaCl, 4.0 ml of 0.005 M MgCl, 8.0 ml of 0.5 M Tris-HCl (pH 7.8), 10.0 ml of nitroblue tetrazolium (1 mg/ml) and

1.0 ml of phenazine methosulfate (1 mg/ml) (Towle, 1977, pers. comm.).

When the gels were removed from the stain, a hypodermic needle coated with india ink was stabbed into the gel marker dye to permanently designate its position and the length of the marker dye migration (MD_2) was recorded. All species except S. m. barbouri had the marker dye designated with india ink. Gels were stored in 7% acetic acid where they expand due to the uptake of water (Gordan, 1969). After gel expansion, the marker dye migration length (MD_1) was again recorded. Measurements of each gel were recorded for the length of each LDH isozyme band migration and band width. All measurements were taken with a micrometer.

Values were calculated for the coefficient of LDH band migration (R_f) by dividing LDH band migration by the marker dye migration. The S. m. barbouri sample had no MD_1 values and all calculations were done using MD_2, therefore the LDH migration is designated R_{f2}. Calculations for the other species were with MD_1, and their LDH migration is recorded as R_{f1}, a more accurate value than R_{f2}. Bands in different tissues with similar R_f values are considered to be the same isozyme. Bands were labeled by designating the most anodal band in heart muscle as LDH-1 and the most cathodal band in body epaxial muscle as LDH-5.

A one-way analysis of variance test (ANOVA) was used to detect differences in LDH band width between tissues and to detect differences in R_f values between tissues. ANOVA tests were then subjected to Duncan's new multiple range test at the 0.05 level of confidence to determine which groups differed significantly from each other. All calculations and statistics were processed by Statistical Package for the Social Science (SPSS) computer programs (Nie, et al., 1975).

RESULTS

Diagrams of muscle LDH isozyme patterns (electrophoretic zymograms) indicate that vibratory muscle LDH patterns resemble body epaxial muscle, not heart muscle (Figures 1-5). Figures 6 through 10 show 95 percent confidence intervals for the mean R_f values of LDH bands. Natrix taxispilota (Figures 5 and 10) has all five LDH isozymes in tail muscle, body epaxial muscle, liver and kidney, whereas S. m. barbouri (Figures 3 and 8) has all five LDH isozymes in heart muscle, body epaxial muscle and kidney. Only 4 LDH isozymes are present in C. adamanteus (Figure 1), C. h. horridus (Figure 2) and A. piscivorus (Figure 4) in all tissues except heart muscle and blood, which have less than 4 LDH isozymes. It is proposed that the missing LDH isozyme in these tissues is LDH-4 because of the wide gap between the LDH-5 and LDH-3 95% confidence intervals shown in Figures 6, 7 and 9. If LDH-4 were present, it would be positioned between LDH-5 and LDH-3.

Individuals of all species had LDH-1 bands in blood. One S. m. barbouri had both LDH-1 and LDH-2 bands in blood (Figure 3).

Heart muscle isozymes consist of LDH-1, LDH-2 and LDH-3 in C. adamanteus (Figure 1), C. h. horridus (Figure 2) and A. piscivorus (Figure 4). The heart LDH pattern of

S. m. barbouri (Figure 3) showed all 5 LDH isozymes, whereas the heart LDH pattern of N. taxispilota (Figure 5) showed only LDH-1 and LDH-2. In all species investigated, LDH-1 was the predominant band in heart muscle, whereas LDH-5 was the predominant band in vibratory muscle, tail epaxial muscle and body epaxial muscle.

The predominant band in liver for C. adamanteus (Figure 1) and C. h. horridus (Figure 2) was LDH-5. Isozyme band intensity of liver resembles body epaxial muscle intensity in C. adamanteus.

There is a general resemblance between tissue LDH patterns in C. adamanteus, C. h. horridus and A. piscivorus. Using LDH band widths as a partial indicator of LDH isozyme quantity, all three species show a significant difference in band widths between vibratory and body epaxial muscles for LDH-3 (Tables 3, 5 and 9). Vibratory muscle LDH-3 bands are wider than those of body epaxial muscle. The vibratory muscle and body epaxial muscle LDH-3 bands appear near equal in intensity in A. piscivorus and C. h. horridus, but in C. adamanteus vibratory muscle LDH-3 is more concentrated than that of epaxial muscle (Figure 1).

There was no significant difference between vibratory and body epaxial muscle LDH band widths for LDH-2 and LDH-5 in A. piscivorus (Table 9), for LDH-1 in C. h. horridus

(Table 5), for LDH-5 in C. adamanteus (Table 3), for LDH-3, LDH-4 and LDH-5 in S. m. barbouri (Table 7) and for all 5 LDH isozymes in N. taxispilota (Table 11). There was a significant difference between vibratory and body epaxial muscle band widths for LDH-1, LDH-2 and LDH-3 in C. adamanteus (Table 3) and for LDH-2, LDH-3 and LDH-5 in C. h. horridus (Table 5). Both have vibratory muscle LDH-2 and LDH-3 bands wider than those of body epaxial muscle, however C. adamanteus LDH-1 and C. h. horridus LDH-5 vibratory muscle bands are thinner than those of body epaxial muscle.

Some LDH bands were too small to measure their exact widths and therefore were recorded as shadows: vibratory muscle LDH-2 and heart LDH-3 bands of S. m. barbouri (Figure 3); vibratory muscle LDH-1 of A. piscivorus (Figure 4); and tail and body epaxial muscle LDH-4 of N. taxispilota (Figure 5). Vibratory LDH-1 in S. m. barbouri was missing, but body epaxial muscle LDH-1 was present.

There were no significant differences in all tissues in R_f values for LDH-3 in C. adamanteus (Table 12), for LDH-2, LDH-3 and LDH-5 in C. h. horridus (Table 14), for LDH-5 in S. m. barbouri (Table 16), for LDH-2, LDH-3 and LDH-5 in A. piscivorus (Table 18) and for LDH-3 in N. taxispilota (Table 20). For all species there were no significant differences between vibratory and epaxial R_f values (Tables

13, 15, 17, 19 and 21) except for LDH-4 R_{f2} in S. m. barbouri (Table 17). The greatest variability in R_f values occurred in the R_{f2} values of S. m. barbouri and is indicated by the large 95% confidence intervals in Figure 8.

DISCUSSION

The tissue distribution of LDH isozymes in snakes and other vertebrates appears to be homologous. Each tissue has characteristic LDH patterns and relative amounts of isozymes. The amount of isozyme present reflects the equilibrium of synthesis and degradation for each of the A and B subunits (Markert and Ursprung, 1962; Markert, 1968; Fritz, et al., 1969; Markert and Masui, 1969; Markert, et al., 1975). Throughout the vertebrates, including the snakes used in this study, the A subunits predominate in white skeletal muscle and B subunits predominate in heart muscle (Schwantes, 1973; Markert, et al., 1975). This uniformity of subunits implies that they perform specific metabolic actions characteristic of each tissue (Markert, et al., 1975).

The homologies of A and B subunits in all vertebrates is based on theories of gene evolution. The hypothesis was proposed that most proteins have evolved by gene duplication from an originally small number of "ancestral genes" (Bridges, 1935; Stephens, 1951; Weltman and Dowben, 1973; Watts and Watts, 1968; Markert, et al., 1975). The ancestral LDH molecule in vertebrate evolution was coded for by a single gene (A) and produced a homotetramer with actions similar to the A_4 (LDH-5) isozyme of skeletal muscle. The A gene duplicated, resulting in two A-like genes which

diverged by mutation to produce two different genes, A and B. It is believed that the two genes diverged early in vertebrate evolution because of all the vertebrates investigated to this date, the only vertebrates which do not have two genes coding for LDH polypeptides are the agnathans Petromyzon marinus (sea lamprey) and Lampetra lamottei (American brook lamprey) (Markert, et al., 1975).

Mammals and birds characteristically have a two-gene, five-isozyme system. All other vertebrates also have at least these two genes coding for LDH but unrestricted subunit association does not always occur (Markert, et al., 1975). The present study has revealed that in the species of snakes, C. adamanteus, C. h. horridus and A. piscivorus, only four isozymes occur, indicating a case of restricted subunit association. The four isozyme pattern is common in fish and has been observed in some amphibians and lizards (Markert, 1968; Whitt, 1970a, 1970b; Markert and Faulhaber, 1965; Markert, et al., 1975). These same four-isozyme LDH patterns also occur in many sharks, e.g. Carcharhinus springeri, the reef shark. The LDH homopolymers (A_4 and B_4) of the reef shark have been isolated, then dissociated and allowed to recombine. The result always produced two intermediate heteropolymers of LDH isozymes. This indicates that all sharks have two genes for LDH, but in some the association

of the corresponding subunits produces only two heteropolymers and not the anticipated three (Markert, et al., 1975). In the present study the missing heteropolymer was determined to be A_3B_1 (LDH-4). This is the first evidence presented for a "four-isozyme" pattern occurring in Crotalid snakes. A previous study has shown the same four-isozyme pattern, missing the heteropolymer A_3B_1 in the South American Colubrid snake, Dryadophis melanolomus (Markert, 1968).

It has been proposed by Markert, et al., (1975) that the intersubunit binding sites of the subunits have been altered during evolution to cause specific restricted subunit associations for certain species while all subunit associations of distantly related species still occur. This shows a significant difference between mammals and birds and may indicate a primary difference between the LDH patterns of ectotherms and endotherms. Natural selection has given rise to an enzyme system in endotherms that allows random subunit association, but in ectotherms, the random subunit association has been limited and may have been selected against in some species (Markert, et al., 1975).

Gorman, et al., (1971) with immunological tests, has shown that lizard and snake LDH isozymes are closely related. Antisera from Iguana iguana (iguana) B_4 LDH reacted with LDH isozymes from both lizards and snakes. The lizard LDH antisera

reacted less with crocodilian, bird, turtle and rhynchocephalian LDH isozymes than with that of snakes. Therefore lizards and snakes (Order Squamata) have immunologically similar LDH isozymes and both have some species with the four-isozyme pattern for LDH (Markert, et al., 1975).

The LDH isozymes of the five species of snakes used in the present study and of the thirteen species of snakes used in an investigation by Schwantes (1973) have shown a smaller net negative charge difference between the isozymes than that of mammals. Consequently the snake isozymes migrated more slowly towards the anode and showed less separation than the corresponding mammalian isozymes. In all mammals, nearly all birds, and in most reptiles and amphibians (Balek, 1967; Moyer, 1968; Zinkham, 1968; Holmes, 1973), electrophoresis of LDH isozymes has shown that the B subunit is more negatively charged than the A subunit. Fishes exhibit the greatest variation in relative net charge on the A and B subunits and in some species the charge may be reversed, thus the A subunit is more negatively charged than the B subunit (Markert, 1968; Markert and Holmes, 1969; Whitt, 1970a; Markert and Faulhaber, 1965).

In previous studies the species differed from those of the present investigation. Individuals of the families

Crotalidae and Colubridae have exhibited an LDH system with all five isozymes, but LDH-1 was not found in liver or body epaxial muscle. Crotalus durissus terrificus (Cascabel or South American rattlesnake) has this pattern and is the species most closely related to those in the present study, but only one specimen was examined (Aleksiuk, 1971; Schwantes, 1973). The present study has shown LDH-1 present in all tissues of all species examined, except the vibratory muscle of S. m. barbouri.

The kidney of S. m. barbouri has an erratic LDH pattern. Two of the specimens had only four isozymes, lacking LDH-1. The remaining two specimens had all five isozymes, but the migration of LDH-1 is significantly greater in kidney than in the other tissues used. It may be possible in the specimens with a high LDH-1 migration that a third subunit C, may have been introduced into the homopolymer by molecular hybridization of C with the A and B subunits (Schwantes, 1973; Whitt, 1970b). Since all specimens of this species didn't have a large LDH-1 (B_4) migration in kidney, it may be due to structural modifications of A or B subunits instead of having an additional subunit formed. It is known that mutants at the B locus in several vertebrates can cause the migration of the isozyme to be vastly changed by a single amino acid substitution (Markert, et al., 1975).

Another variation occurred in blood LDH patterns. The LDH found in erythrocytes of different species is known to be quite variable (Everse and Kaplan, 1975), but in the snakes examined in the present study a species variation also occurred. In C. h. horridus all five specimens showed LDH-1 and one specimen of S. m. barbouri had both LDH-1 and LDH-2, whereas in the remaining species some had only LDH-1 present and the rest lacked LDH in blood. A similar result has been found in certain rodents where the expression of the LDH B gene is restricted in erythrocytes. Red blood cells from several inbred mouse strains have low levels of LDH B while other strains have no B subunits in their red corpuscles. Evolutionary studies have also shown that particular families of rodents characteristically display different proportions of B subunits in their erythrocytes, whereas other tissues of these rodents show no such variation and are virtually identical in LDH patterns (Baur and Pattie, 1968; Shows and Ruddle, 1968; Shows, et al., 1969; Engel, et al., 1972; Markert, et al., 1975). The evidence suggests that the expression of the B gene in rodent erythrocytes is specifically controlled by a regulator gene. Therefore the mutational variations and natural selection which occurs for the structural A and B genes, may also effect the regulatory gene (Shows and Ruddle, 1968; Markert, et al., 1975),

possibly resulting in expression or suppression of the B gene in snake erythrocytes. Another possible cause for LDH to appear in blood is from tissue degeneration. When tissue is damaged or degenerating the disrupting cells release their LDH isozymes into the blood. It is common practice to test for LDH in blood for many disease states in humans(Vesell, 1975). Since LDH-1 is found in snake blood it suggests damage to the heart in those specimens, but that is unlikely.

The fundamental difference between vibratory muscle and body epaxial muscle LDH patterns appears to be in the proportions of LDH heteropolymers. As the degree of vibratory activity in snakes increases, the band width of LDH-2 and LDH-3 increases with a concurrent increase in band intensity. Crotalus adamanteus, with the highest degree of vibratory activity, displays the greatest difference between vibratory muscle and body epaxial muscle, whereas S. m. barbouri and N. taxispilota, show the greatest similarity between vibratory muscle and epaxial muscle. In the latter two species it appears that vibratory muscle isozymes are less concentrated than body epaxial muscle isozymes, consequently a correlation exists between muscle activity and LDH isozyme concentration. Highly active muscle exhibits higher concentrations of LDH heteropolymers while less active muscles have lower concentrations of LDH heteropolymers.

Rattlesnake vibratory muscle is a specialized form of epaxial muscle that can be considered analogous to heart muscle. The degree of activity which occurs in these muscles is achieved in some instances by similar enzyme systems (Forbes, 1967; Kerins, 1969), but heart LDH is composed primarily of B molecules, whereas vibratory muscle primarily A molecules. Evidently the metabolic action of vibratory muscle LDH is characteristic of body epaxial muscle. Thus, the high degree of activity in vibratory muscle is not entirely the result of utilizing the aerobic energy pathway as does the heart muscle. The highly vascular nature of the vibratory muscle along with the high concentration of mitochondria probably give the muscle it's ability to function for periods of time utilizing aerobic energy pathways (Martin and Bagby, 1973; Pastore, 1967). The predominance of LDH-5 in vibratory muscle provides the muscle with an alternate energy route for anaerobic respiration during periods of sustained vibration when oxygen may not be readily available. In this characteristic vibratory muscle parallels liver (Vesell, 1975) as both tissues exhibit an aerobic environment but possess LDH isozymes for anaerobic respiration.

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Table 1. Species of snakes used for LDH isozyme study.

Species	Number		
	Male	Female	Total
Crotalidae			
Crotalus adamanteus	6	3	9
Crotalus h. horridus	5	0	5
Sistrurus miliarius barbouri	3	1	4
Agkistrodon p. piscivorus	2	1	3
Agkistrodon piscivorus conanti	1	0	1
Colubridae			
Natrix taxispilota	5	1	6

Table 2. Analyses of variance for the mean LDH isozyme band widths for all tissues from Crotalus adamanteus.

A. LDH-1

Source*	df	ss	ms	Fr	Fp
Between Groups	5	1.6900	0.3380	360.79	0.0
Within Groups	227	0.2127	0.0009		
Total	232	1.9027			

B. LDH-2

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0705	0.0176	32.85	0.0
Within Groups	204	0.1095	0.0005		
Total	208	0.1801			

C. LDH-3

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.5378	0.1345	210.94	0.0
Within Groups	210	0.1339	0.0006		
Total	214	0.6717			

D. LDH-5

Source*	df	ss	ms	Fr	Fp
Between Groups	3	0.1063	0.0354	26.40	0.0
Within Groups	171	0.2295	0.0013		
Total	174	0.3358			

*Legend. df, degrees of freedom; ss, sum of squares; ms, mean squares; Fr, F ratio; Fp, F probability.

Table 3. Results of Duncan's new multiple range test for the mean LDH isozyme band width in all tissues of Crotalus adamanteus. Means underscored by the same line do not differ significantly at the 0.05 level of confidence.

A. LDH-1

Tissue*	B	V	E	L	K	H
Mean	0.14	0.15	0.17	0.21	0.21	0.39

B. LDH-2

Tissue*	H	L	E	V	K
Mean	0.12	0.12	0.15	0.16	0.17

C. LDH-3

Tissue*	H	L	E	K	V
Mean	0.13	0.14	0.17	0.21	0.26

D. LDH-5

Tissue*	K	E	L	V
Mean	0.29	0.34	0.35	0.35

*Legend. B, whole blood; E, body epaxial muscle; H, heart muscle; K, kidney; L, liver; T, tail epaxial muscle; V, vibratory muscle.

Table 4. Analyses of variance for the mean LDH isozyme band widths for all tissues from Crotalus h. horridus.

A. LDH-1

Source*	df	ss	ms	Fr	Fp
Between Groups	5	0.8456	0.1691	129.95	0.0
Within Groups	125	0.1627	0.0013		
Total	130	1.0083			

B. LDH-2

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0057	0.0014	3.90	0.009
Within Groups	43	0.0157	0.0004		
Total	47	0.0213			

C. LDH-3

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0810	0.0203	10.58	0.0
Within Groups	67	0.1283	0.0019		
Total	71	0.2093			

D. LDH-5

Source*	df	ss	ms	Fr	Fp
Between Groups	3	0.1250	0.0417	20.79	0.0
Within Groups	62	0.1243	0.0020		
Total	65	0.2493			

*See Legend, Table 2.

Table 5. Results of Duncan's new multiple range test for the mean LDH isozyme band width in all tissues of Crotalus h. horridus. Means underscored by the same line do not differ significantly at the 0.05 level of confidence.

A. LDH-1

Tissue*	V	B	E	K	L	H
Mean	0.13	0.14	0.15	0.21	0.22	0.36

B. LDH-2

Tissue*	E	L	H	V	K
Mean	0.10	0.10	0.11	0.12	0.12

C. LDH-3

Tissue*	H	E	K	V	L
Mean	0.13	0.17	0.19	0.21	0.30

D. LDH-5

Tissue*	K	L	V	E
Mean	0.21	0.23	0.28	0.32

*See Legend, Table 3.

Table 6. Analyses of variance for the mean LDH isozyme band widths for all tissues from Sistrurus miliarius barbouri.

A. LDH-1

Source*	df	ss	ms	Fr	Fp
Between Groups	3	0.3129	0.1043	103.48	0.0
Within Groups	25	0.0252	0.0010		
Total	28	0.3381			

B. LDH-2

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0131	0.0033	3.48	0.019
Within Groups	34	0.0326	0.0010		
Total	38	0.0458			

C. LDH-3

Source*	df	ss	ms	Fr	Fp
Between Groups	3	0.0050	0.0017	1.62	0.196
Within Groups	48	0.0496	0.0010		
Total	51	0.0546			

D. LDH-4

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0470	0.0117	3.15	0.023
Within Groups	46	0.1716	0.0037		
Total	50	0.2186			

Table 6, cont.

E. LDH-5

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.3833	0.0958	112.73	0.0
Within Groups	52	0.0442	0.0008		
Total	56	0.4275			

*See Legend, Table 2.

Table 7. Results of Duncan's new multiple range test for the mean LDH isozyme band width in all tissues of *Sistrurus miliarius barbouri*. Means underscored by the same line do not differ significantly at the 0.05 level of confidence.

A. LDH-1

Tissue*	K	E	B	H
Mean	0.11	0.12	0.13	0.33

B. LDH-2

Tissue*	H	B	E	K	L
Mean	0.10	0.10	0.13	0.15	0.16

C. LDH-3

Tissue*	E	V	L	K
Mean	0.13	0.13	0.14	0.15

D. LDH-4

Tissue*	H	E	K	V	L
Mean	0.11	0.14	0.15	0.15	0.21

E. LDH-5

Tissue*	H	K	V	E	L
Mean	0.11	0.16	0.27	0.27	0.38

*See Legend, Table 3.

Table 8. Analyses of variance for the mean LDH isozyme band widths for all tissues from Agkistrodon piscivorus.

A. LDH-1

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.2456	0.0614	85.64	0.0
Within Groups	70	0.0502	0.0007		
Total	74	0.2958			

B. LDH-2

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0048	0.0012	3.23	0.017
Within Groups	73	0.0271	0.0004		
Total	77	0.0319			

C. LDH-3

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0440	0.0110	32.38	0.0
Within Groups	62	0.0211	0.0003		
Total	66	0.0651			

D. LDH-5

Source*	df	ss	ms	Fr	Fp
Between Groups	3	0.0590	0.0197	19.30	0.0
Within Groups	42	0.0428	0.0010		
Total	45	0.1018			

*See Legend, Table 2.

Table 9. Results of Duncan's new multiple range test for the mean LDH isozyme band width in all tissues of Agkistrodon piscivorus. Means underscored by the same line do not differ significantly at the 0.05 level of confidence.

A. LDH-1

Tissue*	B	L	E	K	H
Mean	0.11	0.14	0.15	0.17	0.28

B. LDH-2

Tissue*	V	E	L	H	K
Mean	0.14	0.15	0.16	0.17	0.17

C. LDH-3

Tissue*	H	E	K	L	V
Mean	0.10	0.16	0.16	0.18	0.19

D. LDH-5

Tissue*	K	L	E	V
Mean	0.13	0.18	0.21	0.21

*See Legend, Table 3.

Table 10. Analyses of variance for the mean LDH isozyme band widths for all tissues from *Natrix taxispilota*.

A. LDH-1

Source*	df	ss	ms	Fr	Fp
Between Groups	5	0.7178	0.1446	73.80	0.0
Within Groups	124	0.2412	0.0019		
Total	129	0.9591			

B. LDH-2

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0504	0.0126	13.06	0.0
Within Groups	109	0.1051	0.0010		
Total	113	0.1556			

C. LDH-3

Source*	df	ss	ms	Fr	Fp
Between Groups	3	0.0046	0.0015	1.79	0.155
Within Groups	86	0.0731	0.0008		
Total	89	0.0776			

D. LDH-4

Source*	df	ss	ms	Fr	Fp
Between Groups	1	0.0004	0.0004	4.65	0.063
Within Groups	8	0.0007	0.0001		
Total	9	0.0012			

Table 10, cont.

E. LDH-5

Source*	df	ss	ms	Fr	Fp
Between Groups	3	0.0201	0.0067	2.05	0.115
Within Groups	74	0.2422	0.0033		
Total	77	0.2623			

*See Legend, Table 2.

Table 11. Results of Duncan's new multiple range test for the mean LDH isozyme band width in all tissues of *Natrix taxispilota*. Means underscored by the same line do not differ significantly at the 0.05 level of confidence.

A. LDH-1

Tissue*	B	L	T	E	K	H
Mean	0.12	0.19	0.20	0.21	0.22	0.37

B. LDH-2

Tissue*	H	E	T	L	K
Mean	0.14	0.18	0.19	0.19	0.20

C. LDH-3

Tissue*	E	L	T	K
Mean	0.18	0.18	0.19	0.20

D. LDH-4

Tissue*	K	L
Mean	0.10	0.11

E. LDH-5

Tissue*	K	E	T	L
Mean	0.25	0.28	0.28	0.29

*See Legend, Table 3.

Table 12. Analyses of variance for the mean R_{f1} values of LDH isozymes in all tissues from Crotalus adamanteus.

A. LDH-1

Source*	df	ss	ms	Fr	Fp
Between Groups	5	0.0030	0.0006	7.25	0.0
Within Groups	97	0.0080	0.0001		
Total	102	0.0110			

B. LDH-2

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0013	0.0003	3.74	0.007
Within Groups	94	0.0081	0.0001		
Total	98	0.0094			

C. LDH-3

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0006	0.0001	1.90	0.118
Within Groups	94	0.0070	0.0001		
Total	98	0.0076			

D. LDH-5

Source*	df	ss	ms	Fr	Fp
Between Groups	3	0.0008	0.0003	3.91	0.012
Within Groups	75	0.0052	0.0001		
Total	78	0.0060			

*See Legend, Table 2.

Table 13. Results of Duncan's new multiple range test for the mean R_{f1} values of LDH isozymes in all tissues of Crotalus adamanteus. Means underscored by the same line do not differ significantly at the 0.05 level of confidence.

A. LDH-1

Tissue*	B	V	L	E	K	H
Mean	0.512	0.521	0.521	0.524	0.526	0.534

B. LDH-2

Tissue*	H	L	K	V	E
Mean	0.483	0.488	0.491	0.492	0.492

C. LDH-3

Tissue*	H	L	V	E	K
Mean	0.453	0.455	0.458	0.458	0.459

D. LDH-5

Tissue*	V	E	K	L
Mean	0.394	0.397	0.397	0.403

*See Legend, Table 3.

Table 14. Analyses of variance for the mean R_{fl} values of LDH isozymes in all tissues from Crotalus h. horridus.

A. LDH-1

Source*	df	ss	ms	Fr	Fp
Between Groups	5	0.0044	0.0009	6.88	0.0
Within Groups	135	0.0173	0.0001		
Total	140	0.0218			

B. LDH-2

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0010	0.0002	1.19	0.322
Within Groups	77	0.0154	0.0002		
Total	81	0.0163			

C. LDH-3

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0022	0.0006	1.93	0.113
Within Groups	84	0.0243	0.0003		
Total	88	0.0265			

D. LDH-5

Source*	df	ss	ms	Fr	Fp
Between Groups	3	0.0002	0.0001	0.66	0.579
Within Groups	72	0.0060	0.0001		
Total	75	0.0061			

*See Legend, Table 2.

Table 15. Results of Duncan's new multiple range test for the mean R_{f1} values of LDH isozymes in all tissues of Crotalus h. horridus. Means underscored by the same line do not differ significantly at the 0.05 level of confidence.

A. LDH-1

Tissue*	V	E	B	L	K	H
Mean	0.508	0.514	0.517	0.520	0.523	0.525

B. LDH-2

Tissue*	V	H	E	L	K
Mean	0.476	0.476	0.479	0.483	0.484

C. LDH-3

Tissue*	V	H	L	E	K
Mean	0.444	0.448	0.448	0.449	0.458

D. LDH-5

Tissue*	K	L	V	E
Mean	0.390	0.392	0.393	0.394

*See Legend, Table 3.

Table 16. Analyses of variance for the mean R_{f2} values of LDH isozymes in all tissues from Sistrurus miliarius barbouri.

A. LDH-1

Source*	df	ss	ms	Fr	Fp
Between Groups	3	0.0147	0.0049	17.02	0.0
Within Groups	32	0.0092	0.0003		
Total	35	0.0240			

B. LDH-2

Source*	df	ss	ms	Fr	Fp
Between Groups	5	0.0074	0.0015	5.31	0.0
Within Groups	54	0.0150	0.0003		
Total	59	0.0224			

C. LDH-3

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0082	0.0020	7.06	0.0
Within Groups	59	0.0171	0.0003		
Total	63	0.0253			

D. LDH-4

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0109	0.0027	6.12	0.0
Within Groups	51	0.0227	0.0004		
Total	55	0.0336			

Table 16, cont.

E. LDH-5

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0017	0.0004	1.66	0.169
Within Groups	63	0.0162	0.0003		
Total	67	0.0179			

*See Legend, Table 2.

Table 17. Results of Duncan's new multiple range test for the mean R_{f2} values of LDH isozymes in all tissues of Sistrurus miliarius barbouri. Means underscored by the same line do not differ significantly at the 0.05 level of confidence.

A. LDH-1

Tissue*	E	H	B	K
Mean	0.613	0.620	0.622	0.666

B. LDH-2

Tissue*	H	V	E	L	B	K
Mean	0.574	0.574	0.585	0.590	0.590	0.605

C. LDH-3

Tissue*	V	H	E	L	K
Mean	0.544	0.548	0.548	0.553	0.576

D. LDH-4

Tissue*	V	L	H	E	K
Mean	0.496	0.509	0.520	0.521	0.537

E. LDH-5

Tissue*	V	E	K	H	L
Mean	0.459	0.460	0.462	0.467	0.473

*See Legend, Table 3.

Table 18. Analyses of variance for the mean R_{f1} values of LDH isozymes in all tissues from Agkistrodon piscivorus.

A. LDH-1

Source*	df	ss	ms	Fr	Fp
Between Groups	5	0.0033	0.0007	3.30	0.009
Within Groups	96	0.0191	0.0002		
Total	101	0.0224			

B. LDH-2

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0004	0.0001	0.55	0.702
Within Groups	78	0.0157	0.0002		
Total	82	0.0162			

C. LDH-3

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0012	0.0003	0.76	0.556
Within Groups	62	0.0245	0.0004		
Total	66	0.0257			

D. LDH-5

Source*	df	ss	ms	Fr	Fp
Between Groups	3	0.0007	0.0002	1.19	0.325
Within Groups	43	0.0080	0.0002		
Total	46	0.0086			

*See Legend, Table 2.

Table 19. Results of Duncan's new multiple range test for the mean R_{f1} values of LDH isozymes in all tissues of *Agkistrodon piscivorus*. Means underscored by the same line do not differ significantly at the 0.05 level of confidence.

A. LDH-1

Tissue*	L	E	V	K	B	H
Mean	0.512	0.515	0.518	0.526	0.526	0.526

B. LDH-2

Tissue*	V	L	H	E	K
Mean	0.476	0.479	0.480	0.480	0.484

C. LDH-3

Tissue*	L	H	E	V	K
Mean	0.432	0.434	0.438	0.442	0.443

D. LDH-5

Tissue*	K	L	E	V
Mean	0.361	0.362	0.369	0.369

*See Legend, Table 3.

Table 20. Analyses of variance for the mean R_{f1} values of LDH isozymes in all tissues from Natrix taxispilota.

A. LDH-1

Source*	df	ss	ms	Fr	Fp
Between Groups	5	0.0012	0.0002	4.29	0.001
Within Groups	126	0.0071	0.0001		
Total	131	0.0083			

B. LDH-2

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0007	0.0002	2.90	0.025
Within Groups	115	0.0072	0.0001		
Total	119	0.0079			

C. LDH-3

Source*	df	ss	ms	Fr	Fp
Between Groups	3	0.0003	0.0001	1.86	0.142
Within Groups	91	0.0053	0.0001		
Total	94	0.0056			

D. LDH-4

Source*	df	ss	ms	Fr	Fp
Between Groups	3	0.0009	0.0003	3.66	0.020
Within Groups	40	0.0032	0.0001		
Total	43	0.0041			

Table 20, cont.

E. LDH-5

Source*	df	ss	ms	Fr	Fp
Between Groups	4	0.0017	0.0004	8.08	0.0
Within Groups	79	0.0041	0.0001		
Total	83	0.0058			

*See Legend, Table 2.

Table 21. Results of Duncan's new multiple range test for the mean R_{fl} values of LDH isozymes in all tissues of Natrix taxispilota. Means underscored by the same line do not differ significantly at the 0.05 level of confidence.

A. LDH-1

Tissue*	E	B	T	K	L	H
Mean	0.532	0.533	0.533	0.534	0.535	0.541

B. LDH-2

Tissue*	H	E	T	K	L
Mean	0.473	0.476	0.478	0.479	0.480

C. LDH-3

Tissue*	E	T	K	L
Mean	0.419	0.421	0.422	0.424

D. LDH-4

Tissue*	T	E	K	L
Mean	0.353	0.354	0.361	0.364

E. LDH-5

Tissue*	H	K	E	T	L
Mean	0.306	0.313	0.313	0.314	0.322

*See Legend, Table 3.

Figure 1. Electrophoretic LDH zymograms of tissues* from Crotalus adamanteus.

*Legend. B, whole blood; E, body epaxial muscle; H, heart muscle; K, kidney; L, liver; T, tail epaxial muscle; V, vibratory muscle; MD_1, marker dye migration after gel expansion; MD_2, marker dye migration before gel expansion; relative band shading indicates relative band concentration.

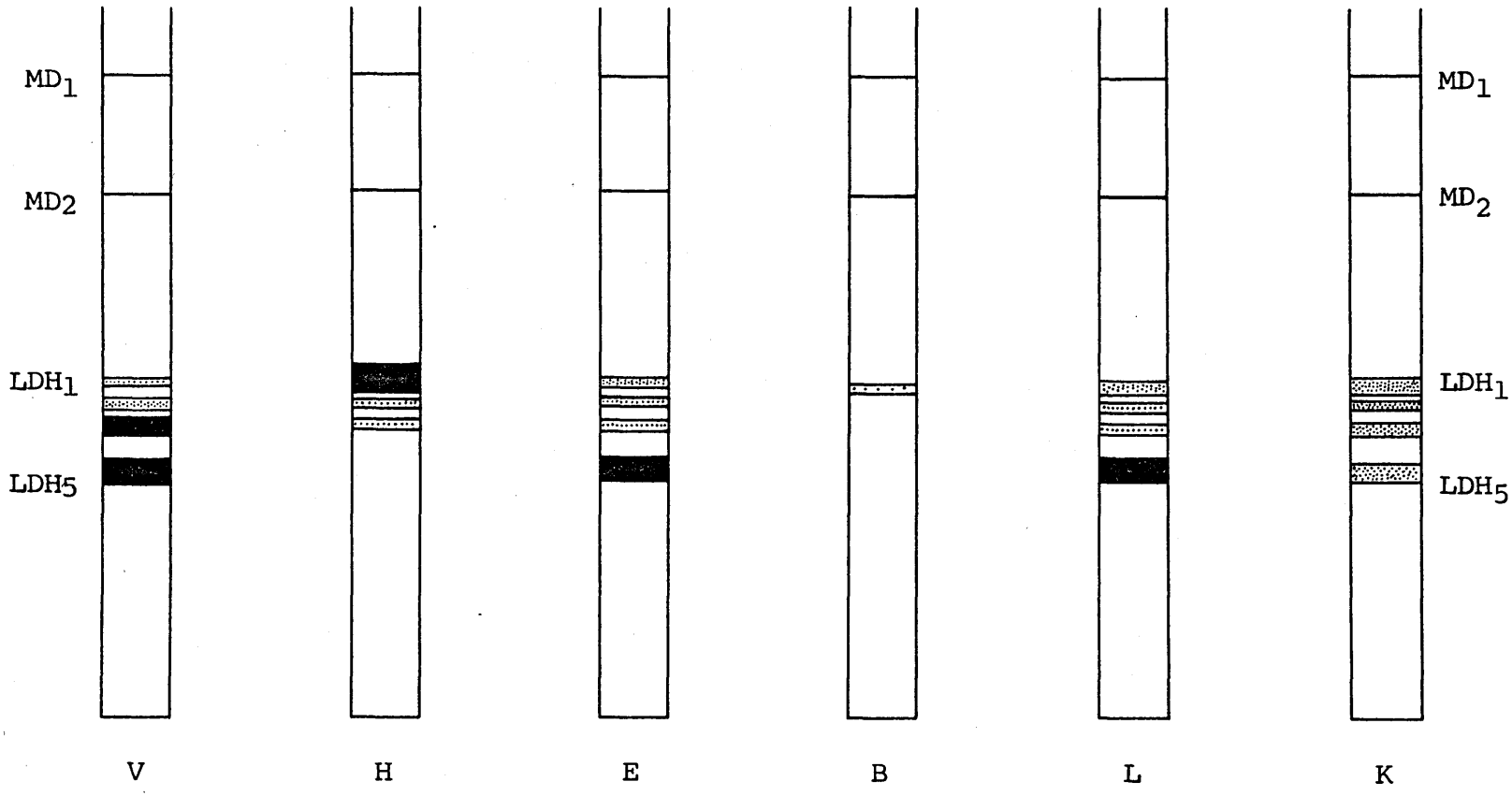

MD1
MD2
LDH1
LDH5
MD1
MD2
LDH1
LDH5
V
H
E
B
L
K
Tissue samples

Figure 2. Electrophoretic LDH zymograms of tissues* from *Crotalus h. horridus*.

*See Legend, Figure 1.

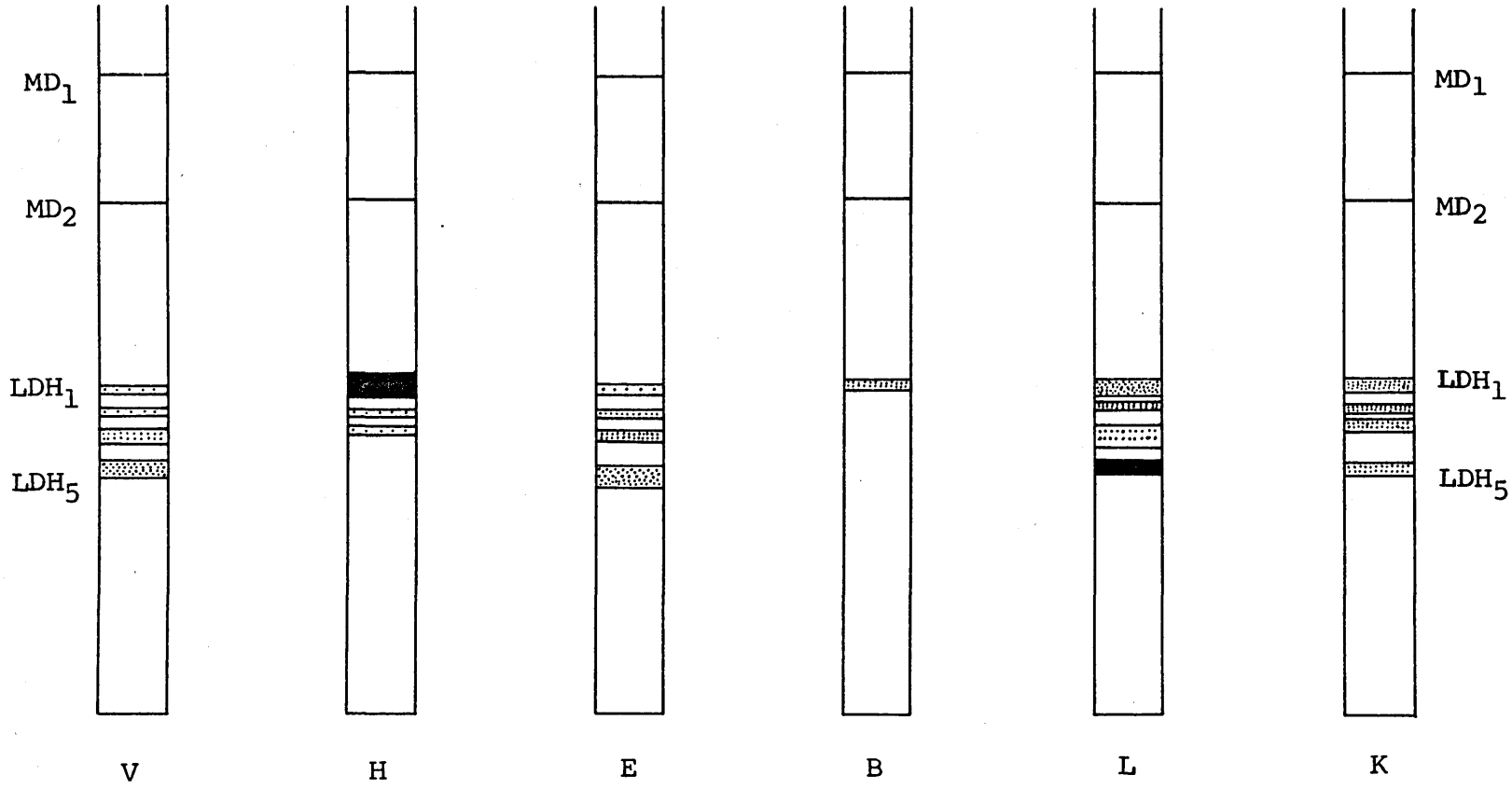

MD1
MD2
LDH1
LDH5
MD1
MD2
LDH1
LDH5
V
H
E
B
L
K
Tissue samples

Figure 3. Electrophoretic LDH zymograms of tissues* from *Sistrurus miliarius barbouri*.

*See Legend, Figure 1.

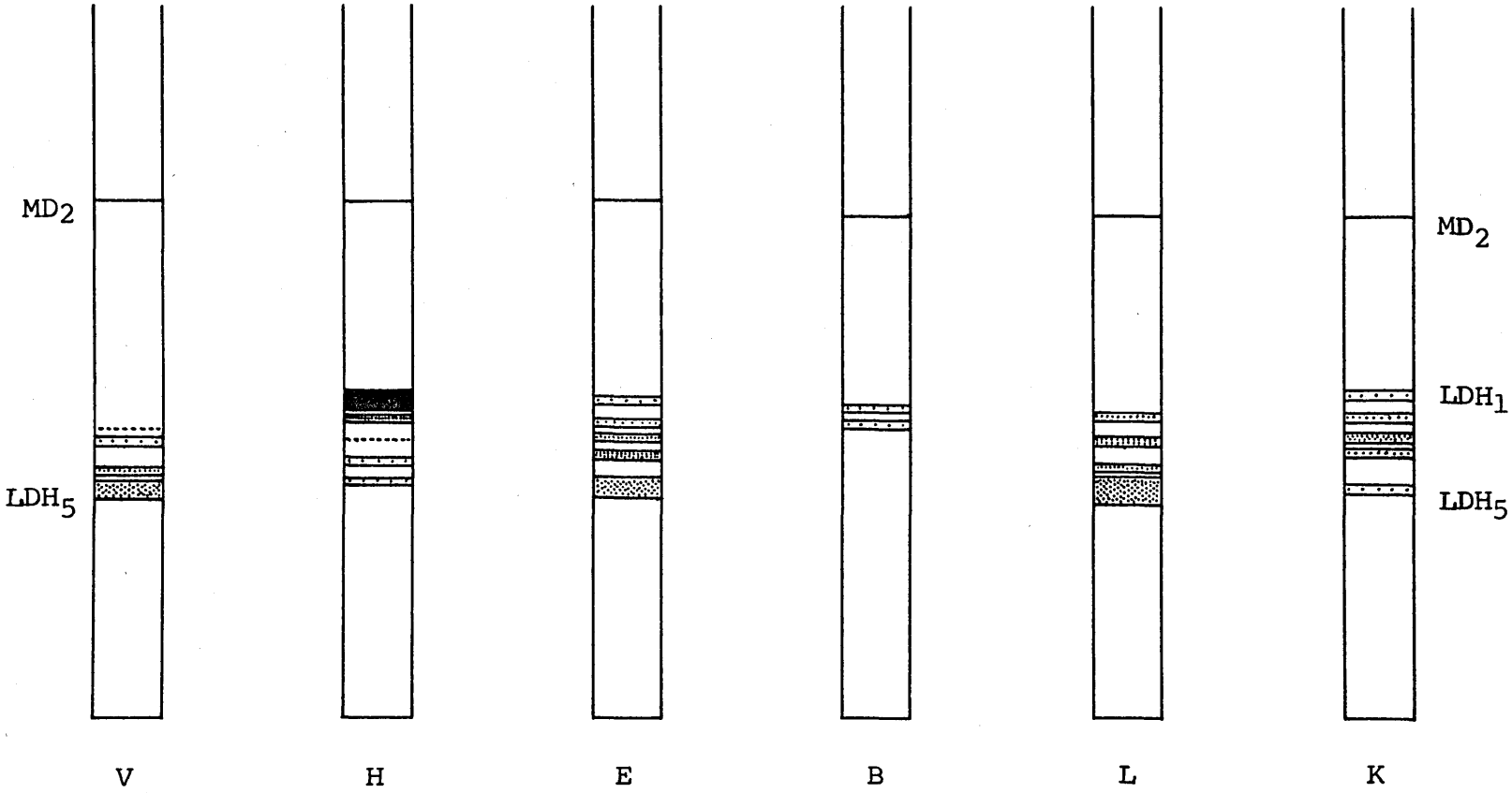

MD2
LDH5
MD2
LDH1
LDH5
V
H
E
B
L
K
Tissue samples

Figure 4. Electrophoretic LDH zymograms of tissues* from *Agkistrodon piscivorus*.

*See Legend, Figure 1.

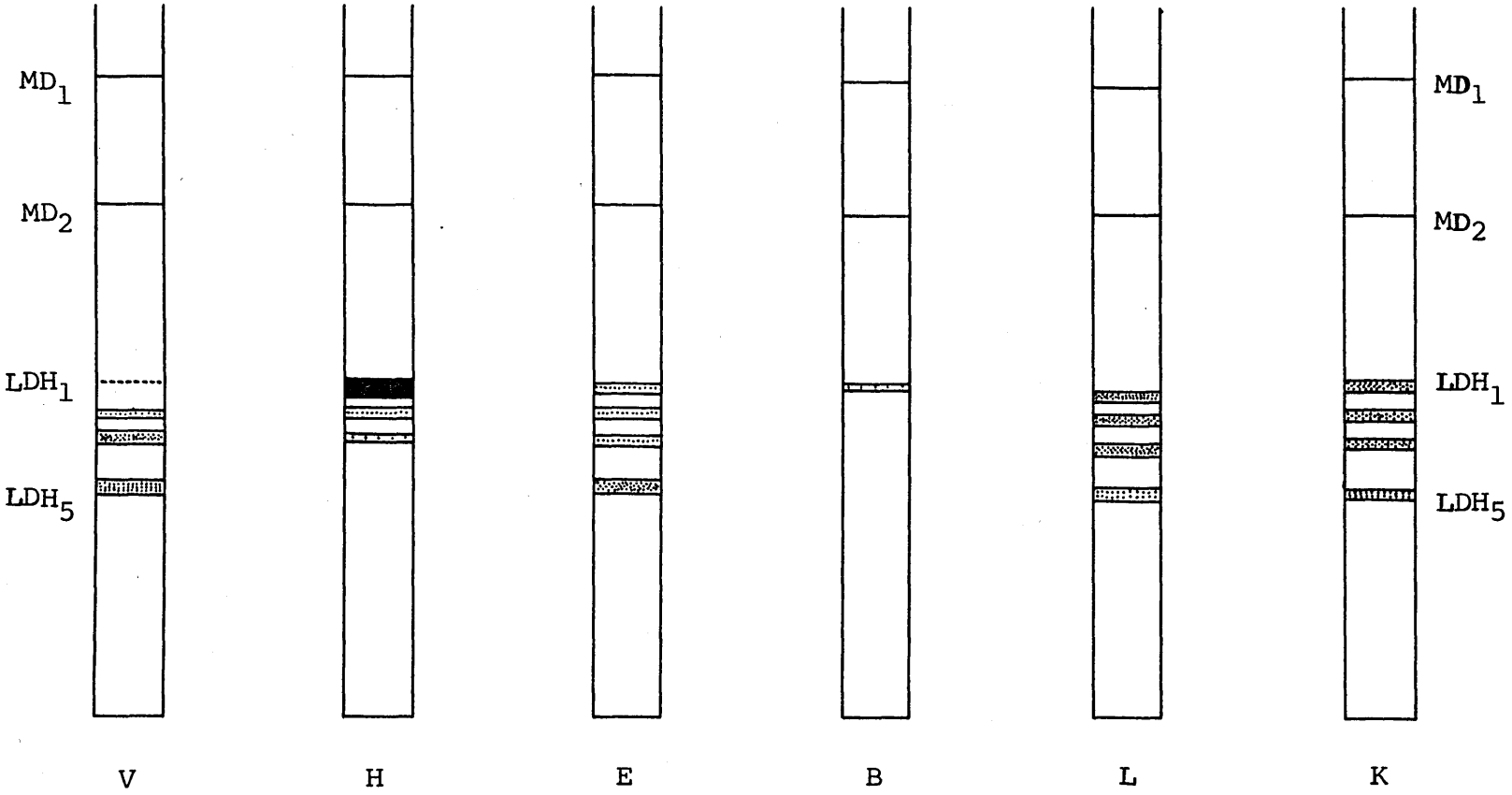
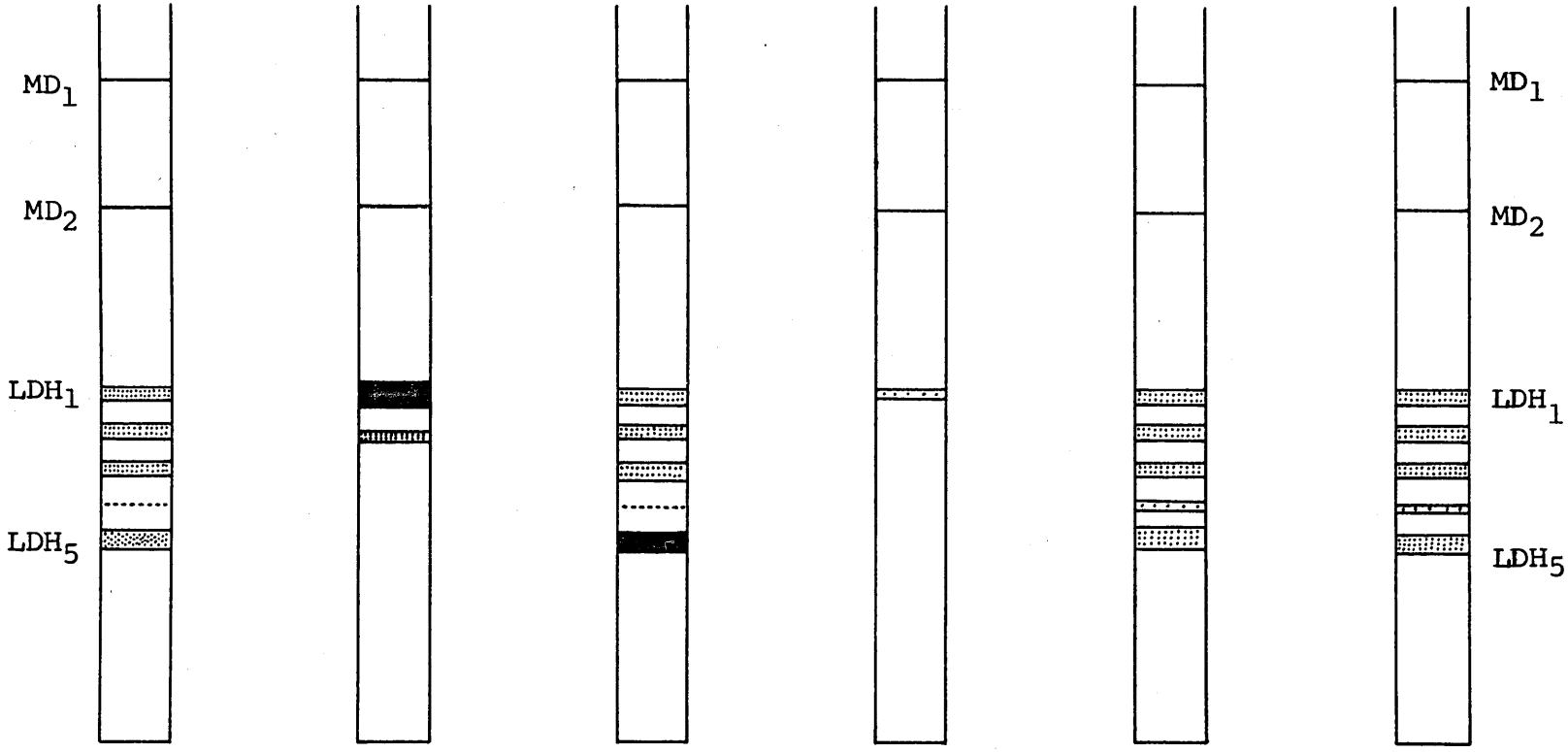

MD1
MD2
LDH1
LDH5
MD1
MD2
LDH1
LDH5
V
H
E
B
L
K
Tissue samples

Figure 5. Electrophoretic LDH zymograms of tissues* from Natrix taxispilota.

*See Legend, Figure 1.



Tissue samples

Figure 6. A comparison of 95 percent confidence intervals for mean R_{fl} values relating similar LDH isozymes from tissues* from Crotalus adamanteus.

*See Legend, Figure 1.

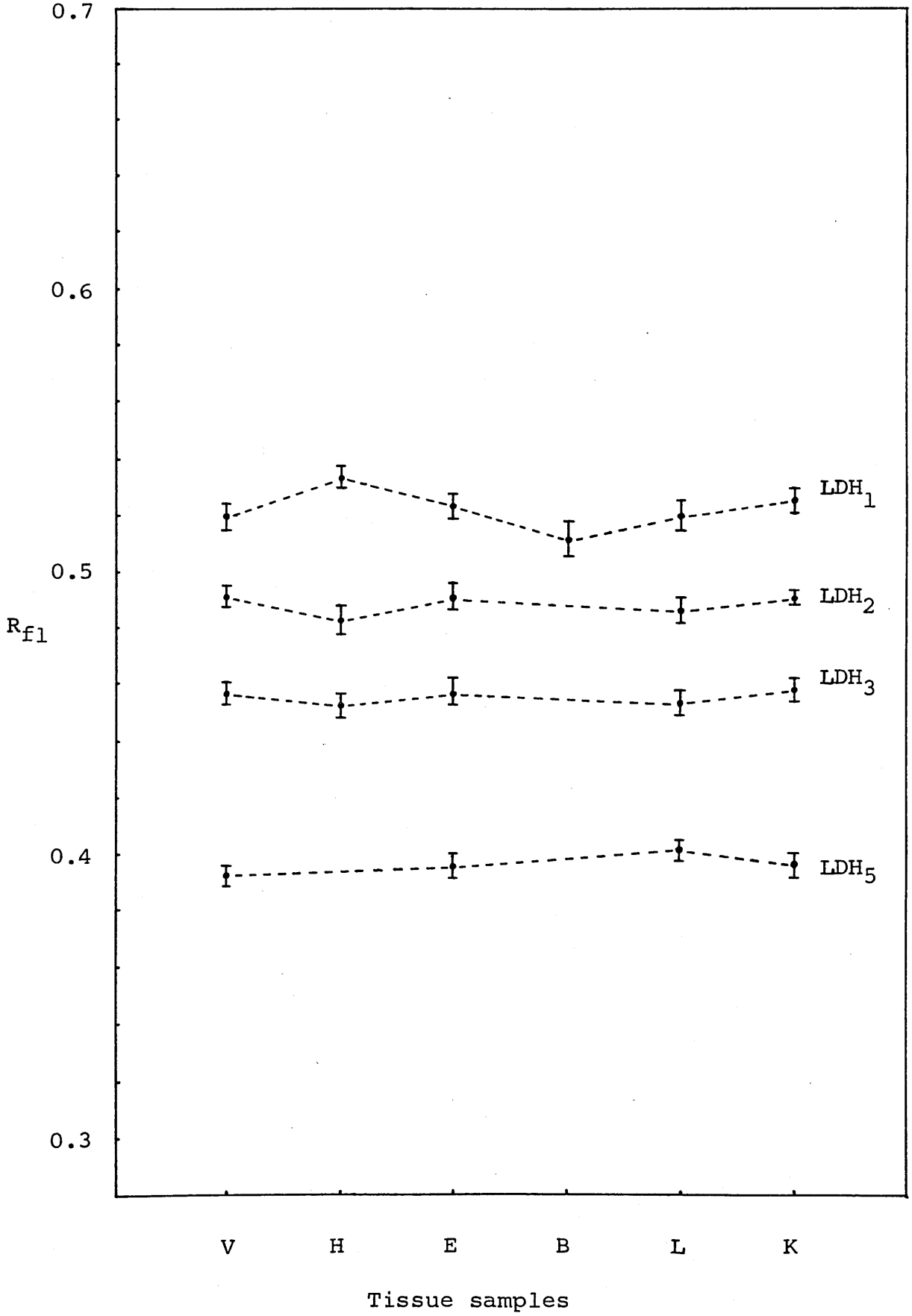

0.7
0.6
0.5
0.4
0.3
R_{f1}
LDH_1
LDH_2
LDH_3
LDH_5
V
H
E
B
L
K
Tissue samples

Figure 7. A comparison of 95 percent confidence intervals for mean R_{fl} values relating similar LDH isozymes from tissues* from Crotalus h. horridus.

*See Legend, Figure 1.

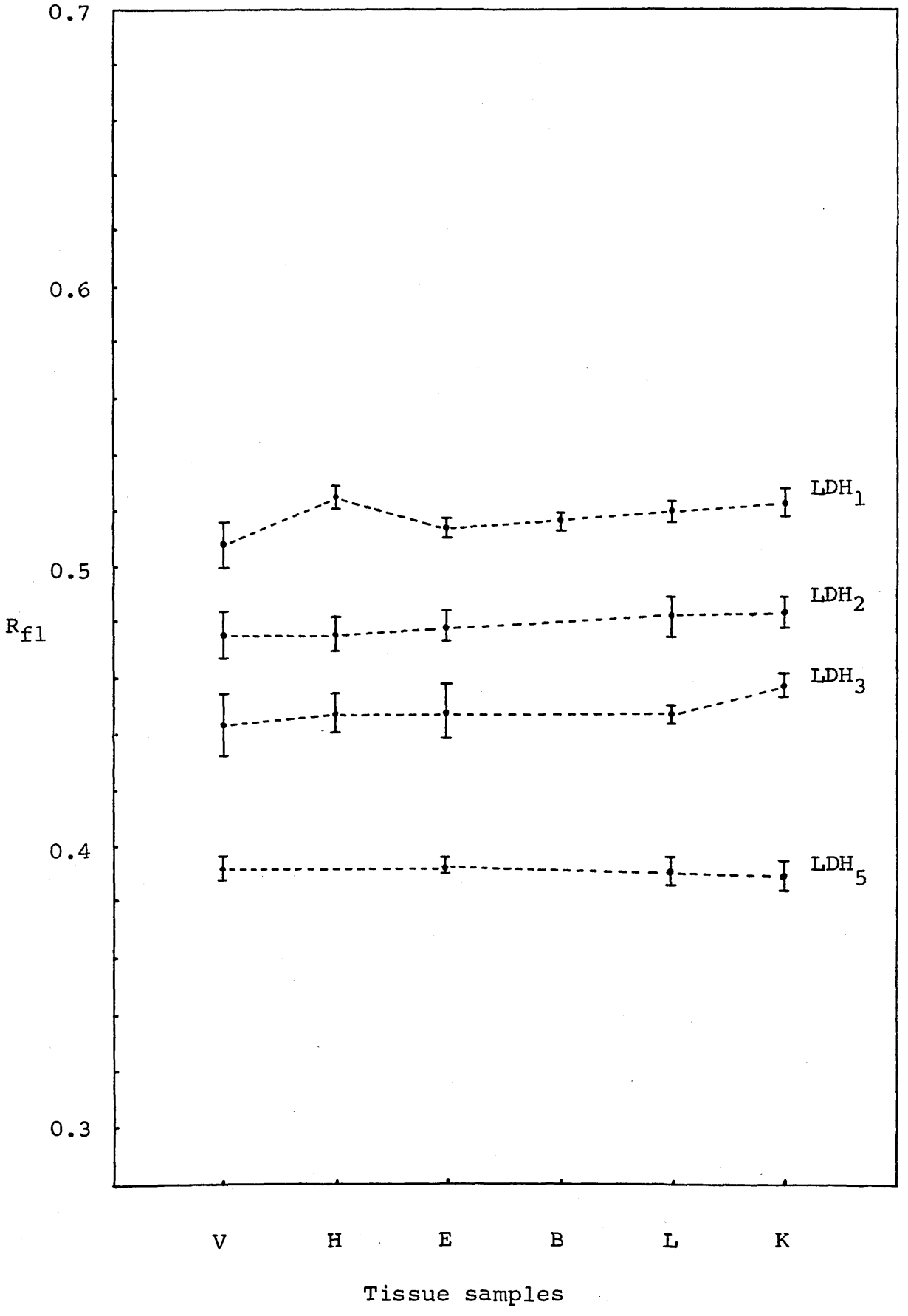

0.7
0.6
0.5
0.4
0.3
R_{f1}
LDH_1
LDH_2
LDH_3
LDH_5
V
H
E
B
L
K
Tissue samples

Figure 8. A comparison of 95 percent confidence intervals for mean R_{f2} values relating similar LDH isozymes from tissues* from Sistrurus miliarius barbouri.

*See Legend, Figure 1.

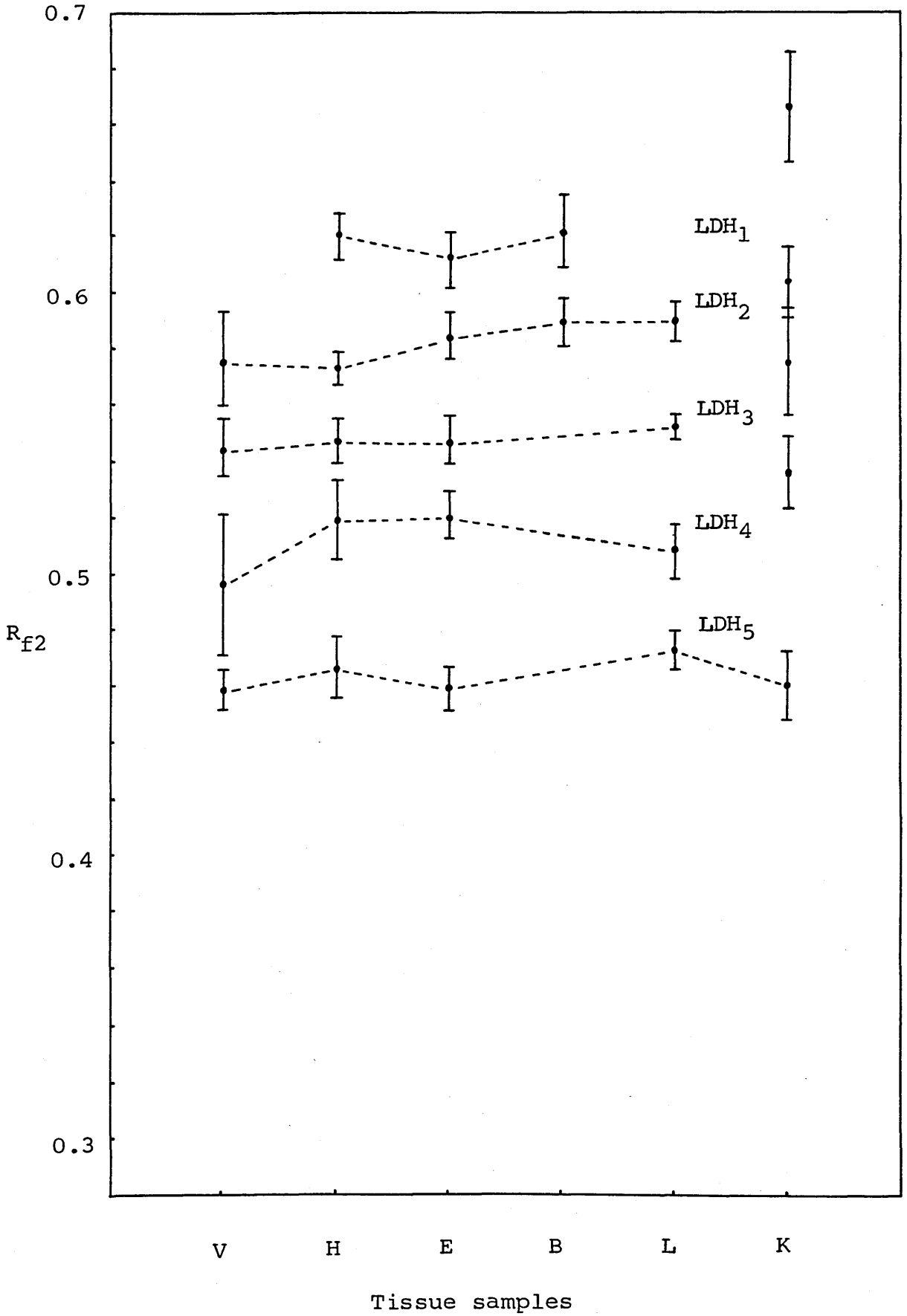

0.7
0.6
0.5
0.4
0.3
R_{f2}
LDH_1
LDH_2
LDH_3
LDH_4
LDH_5
V
H
E
B
L
K
Tissue samples

Figure 9. A comparison of 95 percent confidence intervals for mean R_{f1} values relating similar LDH isozymes from tissues* from Agkistrodon piscivorus.

*See Legend, Figure 1.

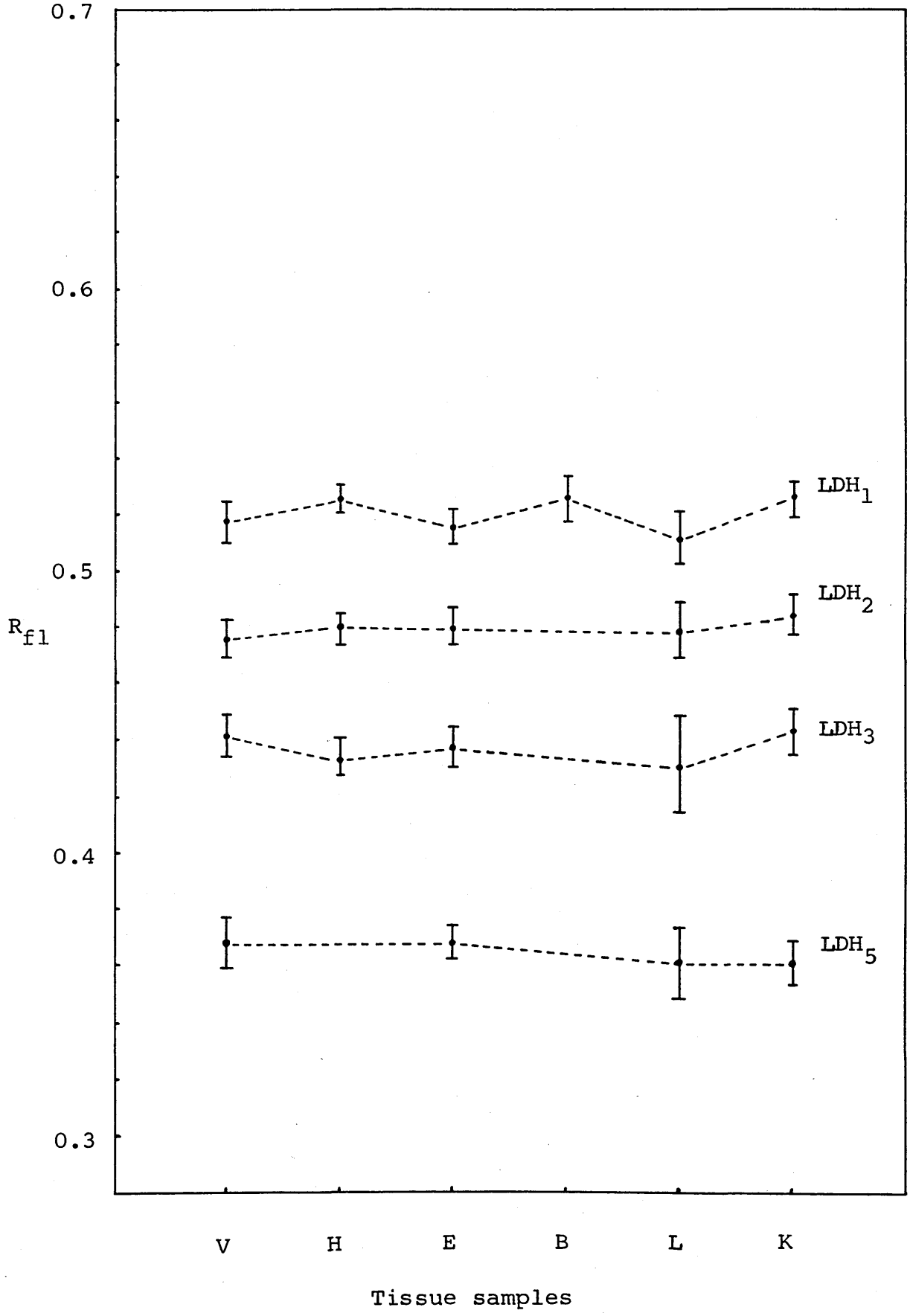
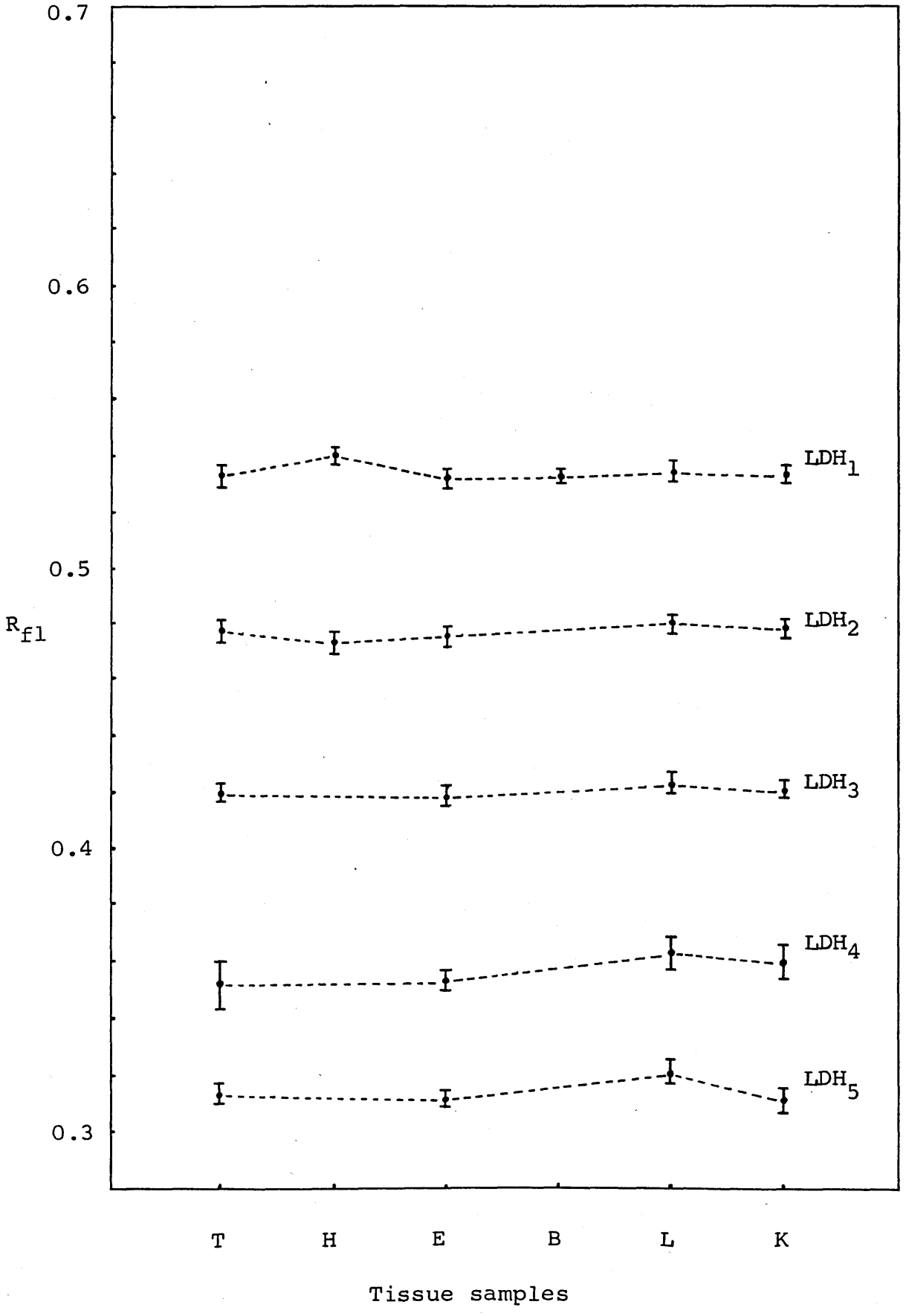

0.7
0.6
0.5
0.4
0.3
R_{f1}
LDH_1
LDH_2
LDH_3
LDH_5
V
H
E
B
L
K
Tissue samples

Figure 10. A comparison of 95 percent confidence intervals for mean R_{fl} values relating similar LDH isozymes from tissues* from Natrix taxispilota.

*See Legend, Figure 1.


0.7
0.6
0.5
0.4
0.3
R_{fl}
LDH_1
LDH_2
LDH_3
LDH_4
LDH_5
T
H
E
B
L
K
Tissue samples

VITA

Cecil Bernard Cross was born December 2, 1952, in Washington, District of Columbia. He received his primary education in the Fairfax County Public School System and graduated from Herndon High School in 1971. He then attended the University of Richmond, Richmond Virginia, where he majored in Biology. While attending the University of Richmond he was elected to the Beta Beta Beta Honorary Biological Society and into the Gamma Sigma Epsilon Honorary Chemistry Society. He received the degree of Bachelor of Science from the University of Richmond in May, 1975. Upon graduation he began graduate work in Biology at the University of Richmond and received a Master of Science degree in August, 1978. He will attend the School of Medicine of the Medical College of Virginia upon graduation.