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Respiratory activity of vibratory, tail epaxial and midbody expaxial muscle in Crotalus horridus, Aqkistrodon contortrix, and Thamophis sirtalis.

James E. Forbes

RESPIRATORY ACTIVITY OF VIBRATORY, TAIL EPAXIAL AND MID-BODY EPAXIAL MUSCLE IN *Crotalus horridus*, *Agkistrodon contortrix* AND *Thamnophis sirtalis*

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RESPIRATORY ACTIVITY OF VIBRATORY, TAIL EPAXIAL AND MID-BODY EPAXIAL MUSCLE IN *Crotalus horridus*, *Agkistrodon contortrix* AND *Thamnophis sirtalis*

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BY

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ABSTRACT

The purpose of this study was to determine if the muscles associated with tail vibration in snakes are physiologically specialized for vibration. Timber rattlesnakes (Crotalus horridus), copperheads (Agkistrodon contortrix) and garter snakes (Thamnophis sirtalis) were used. The copperhead represented a species which vibrates its tail but has no vibratory apparatus comparable to that of the rattlesnake. The garter snake does not vibrate its tail. A comparison of respiratory activities was made between the vibratory and tail epaxial muscle and the mid-body epaxial muscle within and between the three species. Oxygen consumption of teased muscle fibers and muscle homogenate succinic dehydrogenase and cytochrome oxidase activities were measured. The resting QO_2 of teased vibratory muscle fibers of the rattlesnake was found to be significantly higher than the QO_2 of fibers of the tail epaxial muscle of the copperhead which was in turn significantly higher than the QO_2 of tail muscle fibers of the garter snake. Vibratory muscle succinic dehydrogenase and cytochrome oxidase activities were found to be higher in the rattlesnake vibratory muscle than the same enzyme activities of tail epaxial muscles of the other two species. Respiratory enzyme activities in the copperhead were higher than in the garter snake muscle.

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INTRODUCTION

Among the New World snakes there are a number of genera with members that vibrate their tail. However, only the rattlesnake possesses a definite vibratory organ. Other snakes that vibrate their tails are not so specialized. Many proposals have been made in the past as to the possible function of this habit in the rattlesnakes. Klauber (1940) offers the reasonable explanation that the function of the rattle is to warn intruders that might be dangerous to the snake.

Little is known concerning the anatomy of the vibratory musculature or even of snake skeletal muscle in general. Czermak (1857) described the vibratory organ of the rattlesnake as consisting of three masses of muscles (two dorso-lateral and one inferior) that insert on the style and are associated with ten to fifteen of the posteriormost vertebrae. Of the species which vibrate but do not have a definite vibratory organ little is known concerning the anatomy of the musculature involved.

Hess (1963) has described two morphologically different types of muscle fibers in the skeletal muscle of the garter snake (Thamnophis sirtalis) a species which does not vibrate. In one type, each fibril is separated from the neighboring fibrils by sarcoplasmic reticulum giving a punctate appearance under a light microscope. The second type has fibrils that not only appear joined to each other but in general are larger and more irregularly arranged.

Sarcoplasmic reticulum appears to be present in lesser amounts in the second muscle type. No studies have been made to determine if these two morphologically different types of muscle are physiologically different in snakes. However, on the basis of studies of other vertebrates (frog, Kuffler and Vaughan Williams, '53; chick, Ginsborg, '60) it has been shown that the punctate muscles give a twitch when stimulated and that the second type gives a long slow contraction. Hess feels that the two morphologically distinct muscle types are also twitch and slow fibers in the snake. Hess (1965) working with the garter snake found that in the twitch fibers the transverse tubular system and sarcoplasmic reticulum are separated, and that the tubular system and the dilated sacs of the sarcoplasmic reticulum form triads at the level of the junction of the A and I bands. However, in the slow fibers where there is little sarcoplasmic reticulum, the transverse tubular system is completely absent. If the conclusions of Hess regarding the physiological nature of the two morphological muscle types are correct, it is possible that the slow fibers are responsible for maintenance of posture whereas the twitch fibers are functional in body movements and perhaps tail vibration in those species that vibrate.

Several studies have been made on the innervation of skeletal muscle in snakes. Kulchitsky (1924) and Tiegs (1932) described the presence of motor end plates, termi-

nasions en grappe and muscle spindles in Python which does not vibrate its tail. Tiegs also described the presence of the sensory endings of the tendon organs of Golgi in Python. Motor end plates, terminasions en grappe and muscle spindles have also been found in the muscle of the boa constrictor by Hines (1932). Hess (1963 and 1965) has shown motor end plates innervate twitch fibers, and terminasions en grappe innervate slow fibers in the garter snake. These findings suggest that the muscles associated with tail vibration might be innervated by motor end plates while other skeletal musculature is innervated by both end plates and terminasions en grappe. However, a study of these muscles is needed in order for this to be ascertained.

Little is known about the physiology of the vibratory musculature. Only certain members of the Crotalidae have been studied in this respect. Two independent studies have related the velocity of vibration to temperature. Using the prairie rattlesnake (Crotalus v. viridis), Klauber (1940), found a rate of 41.0 cycle per second (cps) at a temperature of 52F and 57.9 cps at a temperature of 73F, an increase of 41% with an increase of 21F. Working with sixteen species and sub-species at temperatures between 65F and 75F the velocity of vibration was seldom above 60 cps or below 40 cps. The rate of vibration is independent of the weight of the rattle, sex and size of the animal. Chadwick and Rahn (1954) demonstrated

a linear relation between temperature and the rate of vibration for c. viridus. In addition, they stated that the movement of the tail is accomplished by three large muscle groups on each side and that on the basis of action potential determinations, all three muscles on a side contract at the same time. Unless the results can be interpreted as meaning that the dorso-lateral mass and lateral portion of the inferior mass on each side contract at the same time, the exact meaning of this work is not clear as their description of the tail musculature does not correspond to that of Czermak (1857).

Rattle duration to the point of muscle fatigue has not been determined. Perry (1920) has observed a timber rattlesnake (Crotalus horridus) to rattle for one-half hour and G. C. Schaefer, University of Richmond (personal communication) has observed C. horridus to rattle for two hours when continuously disturbed in the laboratory. Schaefer has also observed the copperhead (Agkistrodon contortrix) to vibrate its tail for short periods of time when disturbed.

There is no literature available that concerns the respiratory physiology of snake vibratory muscles except Leftwich, et al. (1967) who showed that the QO_2 of the vibratory muscle was higher than that of the mid-body epaxial muscle for C. horridus. They found that the vibratory muscle had more mitochondria than the mid-body epaxial muscle. The activities of succinic dehydrogenase

and cytochrome oxidase were found to be greater in the vibratory muscle than in the mid-body epaxial muscle when assays were made on mitochondrial cell fractions.

It was the purpose of the present study to determine if the muscles involved with tail vibration were physiologically specialized for vibration and if they were different from the skeletal muscles concerned with other body movement of the animal. Comparisons of the respiratory activities of skeletal musculature associated with tail vibration and body movement of the animal were made between a species which vibrates and has a specific vibratory organ (C. horridus), a species which vibrates but has no particular anatomical structure for vibration (A. contortrix), and a species which does not vibrate (T. sirtalis). According to Brattstrom (1964), C. horridus is one of the most advanced species and A. contortrix the most primitive species of Crotalidae. T. sirtalis is a member of the family Colubridae.

MATERIALS AND METHODS

A. Collection and Maintenance of Animals

Ten rattlesnakes, eight copperheads and nine garter snakes were used in the study. Their mean body weights and snout-vent lengths were 313.1gm, 1047.0mm; 116.6gm, 614.0mm; 61.4gm, 666.0 mm, respectively (Table 1). The rattlesnakes and copperheads were captured in Susquehanna and Wyoming Counties, Pennsylvania in June 1966. The rattlesnakes were utilized between July and August 1966, and the copperheads were utilized between September and mid-October 1966. The garter snakes were obtained commercially (E. G. Steinhilber & Co., Oshkosh, Wisconsin). The snakes were received in mid-October and utilized from then through December 1966. All snakes were maintained species separate in clean terraria, provided with water.

B. Preparation of Tissue for the Determination of Muscle Fiber Respiration

The animals were immobilized by pithing. The skin was immediately removed from the area of the vibratory muscle of the rattlesnake and from the epaxial muscle at the tip of the tail below the cloacal opening in the copperhead and garter snake. Pieces of muscle were quickly severed from these regions and placed on aluminum foil-covered ice plates. The muscle was designated vibratory muscle in the rattlesnake and tail epaxial muscle in the copperhead and garter snake. The skin was also removed from the mid-body area approximately midway

between the tip of the head and the cloacal aperture and samples of muscle removed and treated identically as the tail muscle. This muscle was designated body epaxial muscle. The muscle tissue was carefully teased with forceps into fibers of not more than 0.5mm in thickness and samples set aside for the determination of dry weight. One hundred milligram samples of teased fibers were rapidly weighed out for studies of oxygen consumption.

C. Determination of Respiration of Resting Muscle Fibers

Oxygen consumption of muscle fibers was determined manometrically using standard Warburg techniques (Umbreit, et al. 1957). One hundred milligram tissue samples were placed in 15ml Warburg flasks containing the following reaction mixture:

0.3ml - sodium succinate (0.5M in Krebs Bicarbonate)
1.1ml - glassed distilled water
0.4ml - cytochrome c ($10X^{-4}$M in Krebs Bicarbonate)
0.2ml - KCN (20% solution placed in center well on a 2X2cm piece of Whatman's No. 1 filter paper. The center well was ringed with Vaseline.)

The Warburg flasks were placed on the manometers and sealed with Vaseline. The manometers were attached to an oxygen manifold in a constant temperature water bath (Precision Scientific Co. Model 13-U-11) and the contents of the flasks oxygenated and temperature equilibrated at 29C for 15 min. Oxygen consumption was measured at 30 min intervals for the body epaxial muscle of all three species and

for the tail epaxial muscle of the copperhead and garter snake. Readings were made at 15 min intervals for the vibratory muscle of the rattlesnake due to its high rate of oxygen consumption. Measurements were made for a period of three hours. The ratio of dry weight to wet weight was determined for each sample and used to calculate the microliters of oxygen consumed per hour per milligram dry weight of muscle tissue. The following equations were used:

$QO_2 = hk$ Where h=change in pressure observed in mm manometer fluid

k=flask constant

$$k = \frac{V_g \frac{273}{T} + V_f a}{P_0}$$

Where V_g=volume of gas phase

V_f=volume of fluid in vessel

P_0=standard pressure of manometer fluid

a=solubility in reaction mixture of gas involved

D. Preparation of Muscle Homogenate for the Determination of Respiratory Enzyme Activity

Ten percent homogenates of each muscle type were made by grinding 0.2gm of tissue in 1.8ml of glassed distilled water in a Potter-Elvehjem glass to glass tissue grinder attached to a power stirrer. The tissue was ground for 10 min with the mortar end of the grinder placed in a beaker of crushed ice. The homogenate was then diluted by

placing 0.5ml of the homogenate in 7.0ml of 0.03M phosphate buffer (pH 7.4).

E. Determination of Homogenate Succinic Dehydrogenase Activity

Succinic dehydrogenase (SDHase) activity was determined spectrophotometrically by the method of Cooperstein, et al. (1950). This assay is based upon the reduction of cytochrome c during the dehydrogenation of succinate to fumarate in the Krebs tricarboxylic cycle. The reaction is as follows:

$$\text{succinate} + \text{cyt c } Fe^{+++} \xrightarrow[\text{dehydrogenase}]{\text{succinic}} \text{fumarate} + \text{cyt c } Fe^{++}$$

Potassium cyanide must be added to the reaction mixture to prevent the reoxidation of the reduced cytochrome c by cytochrome oxidase which is most always present in tissues. The assay is run at a wavelength of 550mμ because of the ease with which reduced cytochrome c can be distinguished from oxidized cytochrome c at this wavelength.

A Beckman DB spectrophotometer was set at a wavelength of 550mμ and the slit width was set on the narrow mode. A blank cuvette was set-up as follows:

0.1ml - muscle homogenate

1.5ml - glassed distilled water

0.8ml - cytochrome c ($1.5X10^{-4}$M plus 0.17M phosphate buffer (pH 7.4) in a 7:4.2 ratio of buffer to cytochrome c)

0.3ml - KCN ($5X10^{-3}$M)

The optical density (O.D.) of the blank cuvette was recorded. An experimental cuvette was set-up as follows:

0.1ml - muscle homogenate

1.2ml - glass distilled water

0.8ml - cytochrome c

0.3ml - KCN

0.3ml - sodium succinate (0.33M)

Immediately upon the addition of the sodium succinate, the cuvette was upended quickly several times to ensure complete mixing of the reagents with the muscle homogenate and placed in the spectrophotometer. Optical density readings were made at 30 sec intervals for a period of three min. The blank was reread and the O.D. recorded. Approximately 0.9mg of sodium hydrosulphite were added to the experimental cuvette which was then upended several times to completely reduce the cytochrome c. The O.D. of the reduced cytochrome c was recorded. The SDHase activity was calculated as follows:

$$\frac{d \log(\text{cyt c})}{d t} = \text{SDHase activity}$$

$$\frac{d \log(\text{cyt c})}{d t} = \frac{\log(OD_{red}-OD_{t1})-\log(OD_{red}-OD_{t2}) \times 1}{t_2-t_1} \frac{}{\text{dry wt}}$$

F. Determination of Homogenate Cytochrome Oxidase Activity

Cytochrome oxidase activity was determined spectrophotometrically according to the method of Cooperstein and Lazarow (1951). This assay is based upon the oxidation of cytochrome c and the passage of electrons to molecular

oxygen. The reaction is as follows:

$$\text{cyt c } Fe^{++} + 1/2\ O_2 \xrightarrow[\text{oxidase}]{\text{cytochrome}} \text{cyt c } Fe^{+++} + H_2O$$

The spectrophotometer was set at a wavelength of 550mμ and the slit set on the narrow mode. An experimental cuvette was set-up as follows:

3.0ml - reduced cytochrome c (prepared by vigorously shaking 0.15ml of sodium hydrosulphite solution (50mg/ml H_2O) in 30ml of $1.5X10^{-4}$M cytochrome c under aspiration for several minutes)

0.04ml - muscle homogenate

Immediately upon the addition of the muscle homogenate, the cuvette was upended quickly several times and the cuvette placed in the spectrophotometer. Optical density readings were made at 30 sec intervals for a period of three min. After the final reading, approximately 0.4mg of potassium ferricyanide was added to the cuvette to completely oxidize the cytochrome c. The O.D. of the oxidized cytochrome c was recorded. The activity of the cytochrome oxidase was calculated as follows:

$$\frac{d\ \log(\text{cyt c})}{d\ t} = \text{cytochrome oxidase activity}$$

$$\frac{d\ \log(\text{cyt c})}{d\ t} = \frac{\log(OD_{t1}-OD_{ox})-\log(OD_{t2}-OD_{ox})}{t_2-t_1} X \frac{1}{\text{dry wt}}$$

G. Statistical Analysis of Data

Comparison of means was made by the Student's t method for correlated data. Student t values were obtained for

each of the three parameters according to Downie and Heath (1965).

RESULTS

In the following experiments where differences exist between species or within a species, all differences are significant at the 1% level of confidence.

A. Respiration of Teased Muscle Fibers

Oxygen consumption of each of the muscle types was expressed as microliters per hour per milligram dry weight (QO_2). Within a species, males and females did not differ for the QO_2 of either body or tail epaxial muscle (Table 5).

The mean QO_2 of the rattlesnake vibratory muscle was 7.53 as compared to a QO_2 of 0.67 for the body epaxial muscle (Table 2). The mean QO_2 values for the tail and body epaxial muscles of the copperhead were 1.76 and 0.56 respectively (Table 2). There was no difference between the QO_2 values of the tail epaxial and body epaxial muscles of the garter snake, both having a mean of 0.63 (Table 2).

When the three muscle types were compared in the three species, a number of differences were found (Table 6). The mean QO_2 of the rattlesnake vibratory muscle ($\bar{X}$7.53) differed from the mean QO_2 of the tail epaxial of both the copperhead ($\bar{X}$1.76) and the garter snake ($\bar{X}$0.63). In addition, the mean QO_2 of the tail epaxial muscle of the copperhead ($\bar{X}$1.76) differed from the mean QO_2 of the garter snake tail epaxial muscle ($\bar{X}$0.63). No differences were found in the mean QO_2 values of the body epaxial muscle between the three species.

B. Muscle Homogenate Succinic Dehydrogenase Activity

Succinic dehydrogenase activity of each of the muscle types was expressed as the change in O.D. times 10^{-4} per minute per milligram homogenate dry weight. Males and females did not differ within a species for the SDHase activity of either body or tail epaxial muscles (Table 5).

The mean SDHase activity of the rattlesnake vibratory muscle was 25.70 as compared to a mean of 2.85 for the activity in the body epaxial muscle (Table 3). The mean SDHase activity of the copperhead tail and body epaxial muscles was 19.80 and 2.09 respectively (Table 3). No difference was found in the SDHase activity of the tail ($\bar{X}$1.31) and body epaxial ($\bar{X}$1.33) muscles of the garter snake (Table 3).

Table 6 presents a comparison of the SDHase activity in the three species. The SDHase activity of the rattlesnake vibratory muscle ($\bar{X}$25.70) did not differ from the SDHase activity of the tail epaxial muscle of the copperhead ($\bar{X}$19.80), but the rattlesnake vibratory muscle SDHase activity ($\bar{X}$25.70) did differ from the SDHase activity of the tail epaxial muscle of the garter snake. Also, the SDHase activity of the tail epaxial muscle of the copperhead ($\bar{X}$19.80) differed from the SDHase activity of the tail epaxial muscle ($\bar{X}$1.33) of the garter snake. When the SDHase activity of the body epaxial muscles of the three species were compared, no differences were found.

C. Muscle Homogenate Cytochrome Oxidase Activity

Cytochrome oxidase activity of each of the muscle types was expressed as the change in O.D. times 10^{-4} per minute per milligram homogenate dry weight. When males and females were compared within a species, no differences were found (Table 5).

The mean cytochrome oxidase activity of the rattlesnake vibratory muscle was 637.4 as compared to a mean of 21.4 for the activity in the body epaxial muscle (Table 4). For the copperhead, the mean cytochrome oxidase activities were 187.7 and 27.6 for the tail and body epaxial muscles respectively (Table 4). The cytochrome oxidase activity of the tail ($\bar{X}$19.1) and body epaxial ($\bar{X}$19.4) muscles did not differ in the garter snake (Table 4).

Comparisons of the cytochrome oxidase activity between the three muscle types in the three species showed a number of differences (Table 6). The activity of the copperhead tail epaxial muscle ($\bar{X}$187.7) was significantly higher than that of the garter snake tail epaxial muscle ($\bar{X}$19.1) and both were significantly lower than that of the rattlesnake vibratory muscle ($\bar{X}$627.4). Comparison of the cytochrome oxidase activity of the body epaxial muscle between the three species showed no differences.

DISCUSSION

With the high velocity of vibration in rattlesnakes found by Klauber (1940) and Chadwick and Rahn (1954), one would expect a large amount of energy to be expended. The expendure of energy should be reflected in the oxygen consumption and the activities of the respiratory enzymes of vibratory muscles as all of these reactions are involved in the production of energy in the form of high energy adenosine triphosphate (ATP). Molecular oxygen accepts electrons (e^-) from cytochrome a3. The reaction is catalyzed by cytochrome oxidase, resulting in the formation of ATP from adenosine diphosphate and inorganic phosphate. Succinic dehydrogenase catalyzes the dehydrogenation of succinate to fumarate. The active site of SDHase which is a flavoprotein can pass e^- directly to the cytochrome chain toward the production of ATP.

Considering the role of oxygen consumption, SDHase and cytochrome oxidase activities in the transformation of energy, it was not surprising to find a high rate of activity for these three factors in the vibratory muscle of the rattlesnake. As the respiratory activity of tail epaxial muscle was higher than that of body epaxial muscle in the copperhead, it appears that this species is also physiologically specialized for tail vibration. The degree of specialization is, however, not nearly as great as that seen in the rattlesnake.

The fact that the respiratory activity of the three

muscle types was the same for both sexes supports Klauber (1940) who found that the rate of vibration in rattlesnakes was not related to sex.

According to Holmes (1937), Dittmer and Grebe (1958) and Vernberg (1954) the QO_2 (ul/hr/mg dry wt) of the skeletal muscles of several vertebrates is: rat 2.3-6.0; frog 0.18-0.24; pigeon 2.1; toadfish 0.004; scup 0.002 and menhaden 0.061. The temperatures at which these determinations were made were: rat, frog and pigeon-37C and toadfish, scup and menhaden-30C. The values presented in the present research were obtained at 29C. It can be seen that the QO_2 of the rattlesnake vibratory muscle is greater than that of rat, frog, pigeon and fish muscle, and the QO_2 of the tail epaxial muscle of the copperhead is greater than that of frog and fish muscle. The QO_2 of the garter snake tail epaxial muscle and the body epaxial muscle of all three species is lower than the QO_2 of rat and pigeon muscle and higher than that of frog and fish muscle.

The works of Schneider and Potter (1943) and Lawrie (1953) have demonstrated that cytochrome oxidase activity is considerably higher than succinic dehydrogenase activity for the muscles of a number of vertebrates. The present study indicates this to be true of snake muscle also. Ogata (1958) described muscles as containing three types of fibers: red, white and medium. The red muscle fibers have greater succinic dehydrogenase and cytochrome oxidase activity than the white fibers while the succinic dehydro-

genase and cytochrome oxidase activities of the medium fibers are between the activities of the red and white fibers. It is suggested that rattlesnake vibratory muscle consists primarily of the red fibers and the tail epaxial muscle of the garter snake and the body epaxial muscle of all three species of white fibers. The respiratory activity of the tail epaxial muscle of the copperhead suggests that the muscle may consist of medium fibers.

It was concluded that on the basis of this study the muscles associated with tail vibration are physiologically specialized and that this is especially true of the vibratory muscle of the rattlesnake. Questions concerning the phylogenetic significance of the different muscle types were not considered as only three species were used and two of these were members of the same family.

SUMMARY

1. The QO_2, SDHase and cytochrome oxidase activities of vibratory muscle differed significantly from mid-body epaxial muscle in the rattlesnake.

2. The QO_2, SDHase and cytochrome oxidase activities of tail epaxial muscle differed significantly from mid-body epaxial muscle in the copperhead.

3. The respiratory activities of the rattlesnake vibratory muscle were greater than the same activities in all other muscle types studied.

4. The QO_2, SDHase and cytochrome oxidase activities of the copperhead tail epaxial muscle were significantly higher than the same activities of tail epaxial and mid-body epaxial muscle in the garter snake.

5. The respiratory activity of the tail epaxial muscle did not differ from mid-body epaxial muscle in the garter snake.

6. Respiratory activities examined were independent of sex in the three species.

LITERATURE CITED

Brattstrom, Bayard H. 1964. Evolution of the pit vipers. Transact. San Diego Soc. Nat. Hist., 13(11):185.

Chadwick, L. E. and Rahn, Hermann. 1954. Temperature dependence of rattling frequency in the rattlesnake Crotalus v. viridis. Science, 119:442.

Cooperstein, S. J., Lazarow, Arnold and Kurfess, Nancy J. 1950. A microspectophotometric method for the determination of succinic dehydrogenase. J. Biol. Chem., 186:129.

Cooperstein, S. J. and Lazarow, Arnold. 1951. A microspectrophotometric method for the determination of cytochrome oxidase. J. Biol. Chem., 189:665.

Czermak, Joh. 1857. Uber den Schallerzeogenden apparat von Crotalus. Zeit. f. wiss. Zool. Bd., 8:294.

Dittmer, Dorothy S. and Grebe, Rudolph M. 1958. Handbook of Respiration. W. B. Saunders Co., Philadelphia. p291.

Downie, N. M. and Heath, R. W. 1965. Basic Statistical Methods. Harper and Row, New York. p138.

Ginsborg, B. L. 1960. Spontaneous activity in muscle fibers of the chick. J. Physiol., 150:707.

Hess, Arthur. 1963. Two kinds of extrafusal muscle fibers and their nerve endings in the garter snake. Am. J. Anat., 113(3):347.

Hess, Arthur. 1965. The sarcoplasmic reticulum, the T system and the motor terminals of slow and twitch fibers in the garter snake. J. Cell Biol., 26:467.

Hines, M. 1932. Studies in the innervation of skeletal muscle. IV. Of certain muscles of the boa constrictor. J. Comp. Neurol., 56:105.

Holmes, Eric. 1937. The Metabolism of Living Tissues. Cambridge Univ. Press, London.

Klauber, Laurence M. 1940. A statistical study of the rattlesnake. Part VII. The Rattle. Occ. Papers San Diego Soc. Nat. Hist., (6):1.

Kuffler, S. W. and Williams, E. M. Vaughan. 1953. Small-nerve junctional potentials. The distribution of small motor nerves to frog skeletal muscle, and the membrane characteristics of the fibers they innervate. J. Physiol., 121:289.

Kulchitsky, N. 1923. Nerve endings in muscles. J. Anat., 58:152.

Lawrie, R. A. 1953. The activity of the cytochrome system in muscle and its relation to myoglobin. Biochem. J., 55(2):298.

Leftwich, F. B., Schaefer, G. C., Turner, J. E. and Forbes, J. E. 1967. Respiration in the vibratory muscle of Crotalus horridus (Reptila: Crotalidae). Unpublished research. University of Richmond.

McDowell, Sammuel Booker T. and Bogert, Charles M. 1954 The systematic position of Lanthanotus and the affinities of the angunomorphan lizards. Bull. Am. Mus. Nat. Hist., 105:1.

Ogata, Tokuro. 1958. A histochemical study of the red and white muscle fibers. Part I and Part II. Acta. Med. Okayamo. 12:216.

Perry, Armstrong. 1920. Crotalus horridus rattles for half an hour. Copeia, 86:85.

Schneider, W. C. and Potter, V. R. 1943. The assay of animal tissues for respiratory enzymes. II. Succinic dehydrogenase and cytochrome oxidase. J. Biol. Chem., 149:217.

Tiegs, O. W. 1932. The innervation of the striated musculature in Python. Aust. J. Exp. Biol. Med. Sci., 9:191.

Umbreit, W. W., Burris, R. H. and Stauffer, J. E. 1957. Manometric Techniques and Tissue Metabolism. Burgess Publishing Co., Minneapolis.

Vernberg, F. John. 1954. The respiratory metabolism of tissues of marine teleosts in relation to activity and body size. Biol. Bull., 106:360.

Table 1

Sex, Body Weight and Body Length

Animal No.	Sex	Body Wt in Gm	Snout-Vent in mm
C. horridus			
1	F	269.7	989.0
2	M	345.8	1017.0
3	M	362.7	1133.0
4	M	366.7	1186.0
5	F	293.0	837.0
6	M	359.7	1199.0
7	M	611.5	1247.0
8	F	304.4	982.0
9	F	267.0	890.0
10	F	311.2	991.0
mean		313.1	1047.0
A. contortrix			
1	M	81.7	661.0
2	F	102.0	687.0
3	F	131.0	794.0
4	F	221.8	840.0
5	M	94.7	655.0
6	M	89.0	674.0
7	F	103.1	750.0
8	M	109.4	680.0
mean		116.6	614.0
T. sirtalis			
1	F	51.3	610.0
2	M	37.2	655.0
3	M	54.5	645.0
4	F	55.2	654.0
5	F	56.4	660.0
6	M	55.0	654.0
7	M	57.0	662.0
8	F	53.8	634.0
9	M	132.5	820.0
mean		61.4	666.0

Table 2

Oxygen Consumption of Teased Muscle Fibers

Oxygen Consumption (ul O_2/hr/mg dry wt)

	C. horridus		A. contortrix		T. sirtalis	
Animal No.	Body Epaxial	Vibratory	Body Epaxial	Tail Epaxial	Body Epaxial	Tail Epaxial
1	0.67	7.89	0.52	1.84	0.62	0.62
2	0.67	7.55	0.58	1.87	0.62	0.62
3	0.77	7.86	0.59	1.99	0.62	0.62
4	0.67	7.39	0.57	1.81	0.62	0.62
5	0.66	7.61	0.58	1.63	0.63	0.63
6	0.66	7.84	0.53	1.65	0.63	0.63
7	0.65	7.50	0.61	1.73	0.64	0.63
8	0.65	7.51	0.55	1.56	0.63	0.62
9	0.62	7.04			0.63	0.63
10	0.64	7.06				
mean$\pm$SD	0.67$\pm$0.03	7.53$\pm$0.45	0.56$\pm$0.23	1.76$\pm$0.12	0.63$\pm$0.01	0.63$\pm$0.12
	$t_{observed}$=10.00 (significant at the 1% level)		$t_{observed}$=9.90 (significant at the 1% level)		$t_{observed}$=0.50 (not significant)	

Table 3

Succinic Dehydrogenase Activity of Muscle Homogenate

SDHase Activity (change in OD units $X10^{-4}$/min/mg dry wt)

	C. horridus		*A. contortrix*		*T. sirtalis*	
Animal No.	Body Epaxial	Vibratory	Body Epaxial	Tail Epaxial	Body Epaxial	Tail Epaxial
1	2.78	26.83	2.09	18.65	1.47	1.44
2	2.66	26.67	1.96	20.49	1.19	1.35
3	3.22	29.21	2.29	20.88	1.32	1.23
4	3.06	27.31	2.26	21.14	1.33	1.46
5	3.00	27.21	1.82	19.47	1.24	1.27
6	2.87	26.94	2.05	19.44	1.36	1.40
7	2.56	24.75	2.20	19.29	1.32	1.36
8	2.62	23.52	2.09	19.38	1.36	1.40
9	2.97	24.10			1.25	1.36
10	2.84	25.37				
mean$\pm$SD	2.85$\pm$0.49	25.70$\pm$1.97	2.09$\pm$0.93	19.80$\pm$0.16	1.31$\pm$0.08	1.33$\pm$0.08

$t_{observed}$=10.00 (significant at the 1% level)

$t_{observed}$=9.42 (significant at the 1% level)

$t_{observed}$=0.03 (not significant)

Table 4

Cytochrome Oxidase Activity of Muscle Homogenate

	Cytochrome Oxidase Activity of Muscle Homogenate (change in OD units $X10^{-4}$/min/mg dry wt)					
	C. horridus		*A. contortrix*		*T. sirtalis*	
Animal No.	Body Epaxial	Vibratory	Body Epaxial	Tail Epaxial	Body Epaxial	Tail Epaxial
1	19.1	618.4	28.6	191.8	18.4	18.7
2	22.0	621.9	28.3	193.9	18.9	18.0
3	23.0	668.6	27.4	191.6	20.0	17.2
4	19.0	646.1	27.7	186.3	19.2	20.4
5	17.2	631.2	26.6	191.0	18.4	19.2
6	30.4	606.5	27.5	183.2	20.5	20.1
7	20.1	617.5	27.0	183.1	19.4	18.6
8	20.7	646.7	28.0	180.5	20.4	19.7
9	21.9	605.4			19.2	20.0
10	20.8	612.1				
mean$\pm$SD	21.4 ± 2.1	627.4 ± 36.1	27.6 ± 0.7	187.7 ± 4.2	19.4 ± 0.7	19.1 ± 1.0
	$t_{observed}=10.00$ (significant at the 1% level)		$t_{observed}=10.00$ (significant at the 1% level)		$t_{observed}=0.15$ (not significant)	

Table 5

A Comparison of Respiratory Activity on the Basis of Sex

Species	Muscle Type	Sex	QO_2*	SDHase**	Cytochrome Oxidase**
			mean±SD	mean±SD	mean±SD
C. horridus	vibratory	M	7.36±0.27[a]	26.73±1.04	637.9±19.0
		F	7.42±0.37	25.41±1.29	616.9± 6.5
	body epaxial	M	0.68±0.04	2.87±0.21	22.9± 2.8
		F	0.65±0.01	2.84±0.11	19.9± 1.4
A. contortrix	tail epaxial	M	1.67±0.08	19.24±0.23	186.6± 4.8
		F	1.85±0.08	20.45±0.33	188.7± 4.0
	body epaxial	M	0.55±0.02	1.76±0.25	27.6± 0.5
		F	0.59±0.01	2.18±0.11	27.6± 0.4
T. sirtalis	tail epaxial	M	0.63±0.01	1.31±0.06	18.8± 1.0
		F	0.62±0.01	1.39±0.05	19.5± 0.6
	body epaxial	M	0.63±0.01	1.29±0.05	19.6± 0.5
		F	0.63±0.01	1.35±0.06	19.1± 0.8

*QO_2 expressed as ul/hr/mg dry wt

**activity expressed as change in O.D.X10^{-4}/min/mg homogenate dry wt

[a]standard deviation

No significant differences exist when the two muscle types are compared in the same species as to sex.

Table 6

Comparisons of QO_2, SDHase Activity and Cytochrome Oxidase Activity of Vibratory, Tail Epaxial and Body Epaxial Muscles Between Species

Muscle Types Compared	Species	QO_2[a] mean±SD	QO_2 t[d]	SDHase[b] mean±SD	SDHase t	Cytochrome Oxidase[b] mean±SD	Cytochrome Oxidase t
vibratory to tail epaxial	C. horridus	7.53±0.45[c]	9.96**	25.70±1.97	1.02*	627.4±36.1	9.99**
	A. contortrix	1.76±0.12		19.80±0.16		187.7± 4.2	
vibratory to tail epaxial	C. horridus	7.53±0.45	9.90**	25.70±1.97	9.99**	627.4±36.1	10.00**
	A. contortrix	0.63±0.12		1.33±0.08		19.1± 1.0	
tail epaxial to tail epaxial	A. contortrix	1.76±0.12	10.00**	19.80±0.16	9.24**	187.7± 4.2	10.00**
	T. sirtalis	0.63±0.12		1.33±0.08		19.1± 1.0	
body epaxial to body epaxial	C. horridus	0.67±0.03[c]	1.01*	2.85±0.49	0.76*	21.4± 2.1	1.03*
	A. contortrix	0.56±0.23		2.09±0.93		27.6± 0.7	
body epaxial to body epaxial	C. horridus	0.67±0.03	0.80*	2.85±0.49	0.70*	21.4± 2.1	1.00*
	T. sirtalis	0.63±0.01		1.31±0.08		19.4± 0.7	
body epaxial to body epaxial	A. contortrix	0.56±0.23	0.75*	2.09±0.93	0.28*	27.6± 0.7	1.43*
	T. sirtalis	0.63±0.01		1.31±0.08		19.4± 0.7	

[a] QO_2 expressed as ul/hr/mg dry wt

[b] activity expressed as change in O.D.X10^{-4}/min/mg homogenate dry wt

[c] standard deviation

[d] student t values

**significant difference at the 1% level

*not significant

VITA

James E. Forbes was born on March 23, 1943, in Norfolk, Virginia. He received his primary education in the Norfolk Public School System and graduated from Maury High School in 1961. After graduating from high school, he entered Old Dominion College in Norfolk, Virginia, with a major in pre-medical study but after becoming interested in physiology during his junior year, he changed his major to biology. While attending Old Dominion College, he served as Vice-President and President of the Pre-Dental-Medical Club and Vice-President of the Biology Club. In addition, he helped to give the Interest Clubs a voice in the Student Government by being instrumental in the formation of the Interest Club Council of the Student Government Association. He received the degree of Bachelor of Science from Old Dominion College in June, 1965. Upon graduation from Old Dominion College, he attended the University of Richmond to work toward the degree of Master of Science. While attending the University of Richmond, he was elected to the Beta Beta Beta Honorary Biological Society. He received a Master of Science degree from the University of Richmond in August, 1967. An D. D. Williams Fellowship has been awarded to him by the Medical College of Virginia, which he will attend in September, 1967, to work toward the degree of Doctor of Philosophy.