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The morphological and function of the metathoracic flexor tibialis muscle of Eurycantha calcarata.

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CONTENTS

1. INTRODUCTION

The use of legs in defensive behaviour is not common in stick insects, but in the male Eurycantha and related genera, the metathoracic legs play an important defensive role, the insect uses the legs to counter-attack against predators. The enlarged metathoracic legs of the male, with their large spines, have been also presumed to protect the less endowed females and immature insects, the males acting as defenders of the colony (Bedford and Chinnick, 1966; Hsiung, 1985; Lea, 1916; Robinson, 1968). The enlarged metathoracic femur of the male E. calcarata bears sharp spines and the third one from the proximal end of the femur is relatively very large. When the tibia is flexed the main tibial and femoral spines interdigitate together. In the male E. calcarata and its close relative, the stick insect E. horrida (Bedford, 1975), any restrained predator tends to be grasped by the metathoracic legs and impaled on the main femoral spine by the tibial flexion. The grasping movements of the females are much weaker as their metathoracic legs are thinner and have less well developed spines. When an adult male Eurycantha, is disturbed its abdomen is raised and curled dorsally and anteriorly, then the metathoracic legs are raised dorsally and extended laterally (Bedford, 1975). If the insects are further stimulated the metathoracic legs are swung together in a grasping motion. Any object which is in their path is caught and then locked within the femur-tibia joint angle by closing the metathoracic legs. Bedford (1975) concludes that the

morphological adaptation and circumstances in which the behavioural responses occur strongly suggests that the behaviour is defensive. However Hsiung (1987) and Clail (1988) observed that the males E. calcarata, particulary when they are mixed with the females, fight with each other. It should be mentioned that Hsiung and Clail's observations have been made in laboratory conditions and may be different to behaviour of the insect in field e.g. if the insect is disturbed by others it may just walk away. If the exhibition and use of the femur with large spines and strong cuticle are purely defensive or perhaps aggressive actions, we need to explain why they are more highly developed in males.

Robinson (1969) suggested that insect defensive adaptations could be divided into two wide functional categories. 1). Primary-defence systems which reduce the possibility of a predator initiating a prey-capture attempt these consist primarily of cryptic behaviour. 2). Secondary defence systems which are employed after the initiation of a prey-capture attempt and reduce the possibility that the attempt will be successful e.g. defensive chemical secretion, death feigning and defensive leg movements. If a prey-capture attempt is made, the secondary defence systems act in two successive phases. First display e.g. raising up the end of the abdomen and striking together of the metathoracic legs. If the attack persists and the insect is seized finally the insect defends himself by active counter attack with the spines of the legs. The defensive behaviour of E. calcarata is composed of both

the above defence systems. The primary defence includes cryptic behaviour e.g. the insect hiding inside hollow tree trunks during day. The secondary defence system is composed of the two phases, mentioned above.

Several previous studies show the correlation between mechanical properties of arthropods legs, their muscles and their behavioural function. Relatively larger and stronger legs will be advantageous in jumping animals, as this will increase acceleration distance (Bennet-Clark, 1977). The movements of the legs of an insect are determined by the structure of the legs themselves and specially the nature of the articulation (Hughes, 1965). The capability to jump is developed to some degree in many groups of insects, however it is most greatly developed in grasshoppers, fleas, flea-beatles and springtails. The jumping animals are characterized by well developed metathoracic legs enable of considerable extension (Hughes, 1965). The most remarkable adaptation of the praying mantid to its raptorial behaviour is its unusually large and mobile prothoracic legs (Copeland and Carlson, 1977; Gray and Mill, 1985). Holling (1964) suggests that the femur spines in mantids have a mechanical function, to form a snare for the trapped prey. Large legs reduce the power output necessary for a jump and the mechanics of the metathoracic femur-tibial joint may affect locomotory performance (Gabriel, 1985).

The correlation between the femur-tibia joint angle and

physiological properties of the muscle which moves the tibia around the articulations has been observed in several previous studies. The acute femur-tibia angle of fleas has considerable effect by permitting a much greater extension to occur during the jump (Hoyle, 1955; Hughes, 1965; Rothschild and Schlein, 1975). Bennet-Clark (1975) examined the correlation between the femur-tibia joint angle and force produced by the extensor tibial muscle of the locust. He found that the force generated by the extensor muscle of the metathoracic leg of the locust is greatest at small femur-tibia joint angles, maximum force recorded at the angle of 30°, of extension of the tibia and falls away to zero at extension angle of about 150°.

Many previous findings show the relationship between behavioural function of the arthropods and the properties of their muscles. Behavioural functions of some insects or crustaceans are performed by fast movement of their limbs. In some of these animals the physiological and mechanical properties of their muscles are capable of producing a high velocity necessary for the fast movement e.g. the fast extension and flexion of the prothoracic leg of the praying mantid (Gray and Mill, 1983). In others, where fast movement cannot be achieved by the physiological and mechanical properties of a single muscle, a mechanism to store energy and release it rapidly to produce the fast movement is necessary e.g. the fast extension of metathoracic legs of the locust in which the energy is stored by cooperation of the metathoracic flexor and extensor muscles. The metathoracic legs and the femur-tibia articulations of the

locust are also well developed which allows the legs to extend to high degree during the jump (Bennet-Clark, 1975; Heitler and Bräunig, 1988).

The flexor tibialis muscle of the E. calcarata is the principal muscle in the defensive behaviour. The muscle should be capable to produce relatively high power output, in order to remain in contraction and keep a caught object between the femur and tibial spine with the high power for extended periods, during the defensive strike, and enables the insect to inflict significant damage on a predator. Whereas in jumping insects e.g. locust the extensor tibialis muscle is the principal muscle and needs to extend the metathoracic legs relatively fast (Heitler and Bräunig, 1988), in praying mantids both the flexor and extensor muscles of the prothoracic limbs are important for prey capture (Gray and Mill, 1983).

The above observations show the correlation between behavioural function of the arthropods and the properties of their muscles. This suggests that, in order to better understand the behaviour of an animal and the significance of physiological and mechanical properties of the muscles to the behaviour of the animal, and also to find how the muscles are used for the behaviour, the properties of the muscles should be examined. A muscle can be studied in a number of ways e.g. by directly investigating the physiological properties of the muscle, but

this does not reveal the range of fibre types. Therefore the histochemical properties of the muscle and the structure of individual fibres should also be examined so that the composition of the muscles is understood.

There are two important ways to determine physiological properties of a muscle (Prosser, 1973). The first is to examine the muscle isotonically, by measuring velocity of contraction and work done by the muscle lifting a load. The second way is to examine the muscle isometrically, by measuring tension produced by the muscle. The examination of muscle isotonically is more significant to predict behaviour of animals. As Pringle (1972) stated, striated muscles have a wide range of velocities which greatly adaptive for specific functions. He also reported that the physiological properties of the muscles of arthropods do not differ qualitatively from those of vertebrates. Also Josephson (1985) stated that the most significant functional capability of a muscle is its ability to shorten against a load and therefore to do work. There are many studies on the isometric contraction properties of insect muscles (Heitler and Bräunig, 1988; Hoyle, 1955; Theophilidis and Burns, 1983; Weis-Fogh, 1956; Wood, 1958) but although there are some studies on isotonic contraction in vertebrate muscles (Close and Luff, 1974; Luff, 1975; Woledge, 1968), I am not aware of any study on insect muscle, other than flight muscle, which measured the velocity or work done by a muscle, when the muscle did external work. Thus there is a need

for more knowledge about the physiological properties of muscles when external work is done.

The relationship between the power developed, the load sustained and the velocity of muscles have been observed (Bennet-Clark, 1975; Hill, 1950). Woledge (1968) has hypothesized that greater curvature of force-velocity relationship of a muscle correlates with greater mechanical efficiency and slower contraction of the muscle. Prosser (1973) reported that, generally muscles with higher speed generate lower tension. The relationship between the velocity of a muscle and the force produced by the muscle has been studied (Wells, 1965).

Previous studies have shown that histochemical examination of enzyme activity and ultrastructural examination of organelles are important techniques to determine muscle fibre types (Barany, 1967; Govind et al, 1986a, Stokes et al, 1979). Histochemical examination of a muscle allows us to study the distribution of fibre types within the muscle, whereas ultrastructural study allows us to investigate individual fibres of a muscle, the two together give a more complete understanding of the muscle composition. In recent years great use has been made especially in the use of histochemical techniques to determine the functional properties of muscle fibres of vertebrates (Ashmore and Doerr, 1971; Close, 1972; Drews and Engel, 1966; Khan et al, 1973). There are few studies on

invertebrates relating histochemical fibre types to either structure or function. The remarkable exceptions are studies on crustacean dimorphic claw muscles (Govind et al, 1981, 1986a, b, 1987; Jahromi and Atwood, 1971; Kent and Govind, 1981; Ogonowski and Lang, 1979; Ogonowski et al, 1980; Rathmayer and Maier, 1987), on abdominal muscle of crustacean (Ogonowski and Lang, 1979) and studies of the locomotory muscles of the cockroach (Morgan and Stokes, 1978; Morgan et al, 1980; Stokes et al, 1979).

The correlation between the intensity of staining for specific enzymes and functional properties of muscle fibre types has been observed (Ashmore and Doerr, 1971; Govind et al, 1986a, b; Khan et al, 1973, Stokes et al, 1979). The oxidative enzymes such as; Nicotine adenine dinucleotide-tetrazolium reductase, NADH-TR, succinic dehydrogenase (SDH) and lactic dehydrogenase (LDH) are indicators for cellular metabolism, these enzymes reflect the utilisation of intermediates in the Krebs cycle and related metabolic pathways (Morgan et al, 1980; Smit et al, 1967; Stokes et al, 1979). Fibres which stain with high intensity for these enzymes are predicted to be fatigue resistant. The staining intensity of fibres for these enzymes depend on density of mitochondria within fibres e.g. highly oxidative fibres which have more mitochondria, stain darkly for NADH-TR. Whereas muscle fibres with fast contraction and rapid fatigue, have much fewer mitochondria and stain poorly for NADH-TR (Nachlas et al, 1958;

Stokes, 1987). High myosin adenosine triphosphatase (ATPase) activity is correlated well with high contraction velocity of muscle fibres (Barany, 1967; Lehman and Szent-gyorgi, 1975; Stokes et al, 1979). The ATPase activity depends upon the myosin filaments density of fibres.

Some previous studies show that the oxidative enzymes staining may also be an indicator to determine the distribution of mitochondria within the muscle fibres. Crustacean slow muscle fibres stain darkly close to the sarcolemma, when they are tested for NADH-TR, which shows that the mitochondria are situated around the periphery of the fibres e.g. in abdominal muscles of crayfish and lobster (Ogonowski and Lang, 1979) and in the closer muscles of fiddler crab (Govind et al, 1986b). Whereas slow muscle fibres of insects e.g. the mesocoxal muscles of the cockroach (Stokes et al, 1979) stain uniformly throughout their cross sectional area for the NADH-TR activity. The mitochondria in insect muscles are scattered through cross section of fibres.

The previous investigations on histochemical properties of muscles show that the distribution and population of fast and slow fibres within vertebrate muscles are different to those of arthropods. In most vertebrate muscles the fast and slow fibres are mixed in a mosaic pattern (Drews and Engel, 1966). By contrast the distribution and population of fibre types in

crustacean and insect muscles are not mixed as they are in vertebrate muscles e.g. in the cutter claw of lobster the majority of fibres are fast and a small ventral area of the muscle is composed of slow fibres (Govind, 1982; Ogonowski et al, 1980). The crusher muscle of the lobster is composed of only slow fibres (Ogonowski et al, 1980). In some insect muscles e.g. mesocoxal and metacoxal muscles of the cockroach the fibres are grouped into homogeneous bundles (Stokes et al, 1979).

Fine structural differences between fast and slow muscle fibres of both vertebrate and invertebrate have been established through broad comparative studies with the electron and light microscope. In crustacean muscles (Huxley, 1965; Jahromi and Atwood, 1967, 1971; Josephson, 1975; Kennedy and Takeda, 1965; Parnas and Atwood, 1966; Wiersma, 1955), in insect muscles (Becht and Dresden, 1956; Edwards, et al, 1956; Hoyle, 1967, 1978; Jahromi and Atwood, 1969b; Smith, 1966) and in vertebrate (Page, 1965; Peachey and Huxley, 1962). The previous findings show that a considerable variation in ultrastructure occurs between different muscles. A great variation is found between ultrastructure of fibres within a single muscle of invertebrates. The structure of vertebrate skeletal muscle, however is relatively uniform in a wide range of muscles.

Papers published to date indicate that fast and slow muscle fibres differ in several ultrastructural criteria. The first

criterion which very strongly correlates with physiological fibre types is sarcomere length slow muscle fibres have longer sarcomere than fast fibres. The sliding-filament hypothesis first stated by Huxley and Niedergerke (1954) proposes that the fibres with longer sarcomere produce more tension than fibres with shorter sarcomeres, and also that the fibres with shorter sarcomere are faster. Sarcomere length differs remarkably amongst different arthropod muscle fibres and between fibres within a single muscle. Crustacean muscles contain sarcomeres of different lengths within the range of 3-14 μm (Franzini-Armstrong, 1970; Pringle, 1972). By contrast in the striated muscle fibres of vertebrate almost all sarcomere lengths are 2-3 μm (Elder, 1975).

The second structural criterion which is strongly related to physiological properties of the muscle fibre types is the density of mitochondria. The muscle fibres which are rich in mitochondria are high oxidative and fatigue slowly, however, those with few mitochondria are low oxidative, fatigue rapidly (Atwood, 1972, Morgan and Stokes, 1978; Smit et al, 1967). The flight muscle of insects is the most metabolically active tissue and has a large amount of mitochondria (Elder, 1975; Smit et al, 1967; Weis-Fogh, 1964).

The third structural criterion which has been found to correlate well with physiological properties of the insect muscle fibres

is the density of sarcoplasmic reticulum (SR) (Cochrane et al, 1972; Huddart and Oates, 1970; Smith, 1962). The correlation between development of the SR and the muscle fibre types has also been found for vertebrates (Grinyer and George, 1969; Page, 1969; Peachey and Schild, 1968). Rosenbluth (1969) describes an extremely fast muscle of the lobster second antenna, where about three quarters of the fibres is occupied by reticular elements. The junction between SR and transverse tubule system (TTS) which form dyads, is typical of insect muscles (Smith, 1966). Occasionally triads have been noted (Cochrane et al, 1972; Hoyle, 1969). Whereas in the vertebrate muscles connection between TTS and SR form triads (Huxley, 1964; Porter and Palade, 1957). The previous observation show that in insect fast muscle there are much more dyads than in slow ones (Atwood, 1972; Hagopian and Spiro, 1967; Huddart and Oates, 1970).

A fourth structural criterion which may be related to physiological properties of the muscle fibres is the ratio of thin to thick filament. Fast muscle fibres of arthropods have a thin to thick filament ratio of 3:1 (Fahrenbach, 1967; Reger and Cooper, 1967) and slow muscle fibres of arthropods have ratio from 5:1 to 6:1 (Franzini-Armstrong, 1970; Huddart and Oates, 1970; Smith, 1966). Whereas in most vertebrate muscles the ratio is 2:1 and the ratio in flight muscles is 3:1 (Huxley and Hanson, 1957; Prosser, 1973; Smith, 1961). Some previous studies also show a correlation between the density of thick filament

and the physiological properties of the muscle fibres (Jahromi and Atwood, 1969b). The difference between the thin to thick filament ratio of the insect muscle fibres and that of vertebrates has been described to be largely due to the density of thin filaments which is much lower in vertebrates (Hagopian, 1966; Hoyle, 1969; Huxley, 1957; Pringle, 1972). The arrangement of the thin filament surrounding each thick filament is related to the ratio of thin to thick filaments. In slow fibres that the ratio is higher the arrangement is less regular (Elder, 1975; Pringle, 1972; Rosenbluth, 1969).

A fifth structural criterion, which weakly correlates with fibre types is the fibres cross sectional area. The correlation has been reported by several previous findings (Elder, 1975; Jahromi and Atwood, 1971; Morgan and Stokes, 1979; Selverston, 1967).

The relationship between pattern and width of Z line and fibre types have been observed by some authors. In arthropods (Cochrane et al, 1972; Franzini-Armstrong, 1970; Huddart and Oates, 1970) and in vertebrate (Page, 1965; Peachey and Huxley, 1962; Gauthier, 1969; Padykula and Gauthier, 1966). The authors show that, the Z line width varies greatly in different types of arthropod muscles fibres. The Z line in slow fibres is thicker than that of fast fibres and it is straighter in fast fibres. The differences in Z line of slow and fast fibres from invertebrates muscles are similar to those of vertebrates.

The previous studies on the correlation between ultrastructure and physiological properties of the muscles, mentioned above, show that sarcomere length, density of mitochondria, sarcoplasmic reticulum and density of thick filaments are strongly correlated with muscle fibre types. However the ratio of thin to thick filament, thin filament density or cross sectional area of the fibres are less significant in predicting physiological properties of fibres.

The behavioural function, defensive strike, of the male E. calcarata. is dependent upon the properties of the metathoracic flexor tibialis muscle. In order to understand the behaviour of the insect and the significance of physiological and mechanical properties of the muscle in the behaviour of the insect, I decided to investigate the physiological properties of the muscle. The main properties to be studied are, contraction velocity, work done and force produced by the muscle of the male, in which the metathoracic legs are used for defensive strike, and those of the female, where the legs are used primarily for locomotion. Some minor physiological properties e.g. tension produced at various femur-tibia joint angle and power output will be investigated. The histochemical capacity e.g. the ATPase activity, and the ultrastructural properties e.g. sarcomere length, density of mitochondria and density of SR of the fibres within the muscles also will be examined, to analyse fibre types and the population and distribution of the

fibres within the muscles. It was also decided to investigate the mechanical properties of the metathoracic leg.

2. MATERIAL AND METHODS

2.1. ULTRASTRUCTURAL EXPERIMENTS

2.1.1. Dissection

Adult males and females of the Eurycantha calcarata, Lucas, 1870, (Cheleutoptera: Phasmatodea: Phasmatidae: Eurycantinae) which were cultured at a constant temperature of 23° C and fed on bramble leaves, were used for dissection of the metathoracic flexor tibialis muscle. For the ultrastructural analyses the muscle was divided longitudinally in the horizontal (dorsal-ventral) plane and the vertical (anterior-posterior) plane to produce four sample regions. Each of the four longitudinal regions were then further divided into three subregions (distal, medial and proximal) to produce a total of 12 sample parts. The 12 parts dissected were as below:

1-Dorsal	distal	anterior	(DDA)
2- "	"	posterior	(DDP)
3- "	medial	anterior	(DMA)
4- "	"	posterior	(DMP)
5- "	proximal	anterior	(DPA)
6- "	"	posterior	(DPP)
7-Ventral	distal	anterior	(VDA)
8- "	"	posterior	(VDP)
9- "	medial	anterior	(VMA)
10- "	"	posterior	(VMP)
11- "	proximal	anterior	(VPA)
12- "	"	posterior	(VPP)

The above abbreviations are used throughout the text to refer to the parts of the muscle sampled and where the text refers to "region" it is referring to the six parts which compose that region.

The pinnate fibres of the flexor muscle attach to the anterior and posterior femoral cuticles and to the central apodeme as shown in figure 10.

Prior to dissection the animal was cooled in the refrigerator for some 5 min, to make it less active. It was then decapitated, the leg was removed by cutting at the coxa-thoracic articulation and the tibia sectioned close to the femur-tibia articulation. For dissection of fibres from the ventral and the dorsal surfaces of the muscle, the ventral cuticle and the dorsal cuticle were removed respectively, by cutting with an abrasive disc and all overlying soft tissues such as the extensor muscle, crural nerve and trachea, were removed to expose the flexor muscle. The dissection was performed in saline (see below) using a dissecting microscope. The femur with intact flexor muscle was placed in a jar of prefixative to ensure that the muscle fibres were fixed at their normal resting length. After approximately 10 min in prefixative the fibres were dissected out and placed in individual jars of prefixative to keep the different muscle samples separate. The fixation was continued as in 2.1.4. below:

2.1.2. Saline

So far a saline has not been developed for Eurycantha. Goodall (1985) measured the blood plasma ion content and found the total osmolarity to be similar to that of Carausius morosus. Two salines have been recommended for Carausius morosus (Weidler and Diecke,1969; Wood,1957) the contents of which are described below:

High Mg^{+2} saline (Wood 1957):

NaCl	20	mM
KCl	20	mM
$MgCl_2$	50	mM
$CaCl_2$	20	mM
Sucrose	100	mM
Hepes	5	mM
PH	7.5	

High Na^{+} saline (Weidler & Diecke 1969):

NaCl	180	mM
KCl	5	mM
$MgCl_2$	25	mM
$CaCl_2$	7.5	mM
Hepes	5	mM
PH	7.4	

Muscle tissue of E. calcarata was dissected using the two salines and fixed in a common fixative. Observations with the

electron microscope revealed that the tissue dissected in high Na^+ saline was better preserved.

2.1.3. Fixative and embedding

The same fixative and embedding techniques were used for both light and electron microscope studies. As no previous studies have been made for the histology of E. calcarata it was necessary to develop a suitable fixation procedure.

Two methods were tested, these were: 1). After Fahrenbach (1967), the tissue was prefixed in 1% glutaraldehyde, 5% formaldehyde in s-collidine buffer with 3% NaCl, 4.5% sucrose and 0.05% $CaCl_2$. The prefixative was buffered at pH 7.4 and tissue was prefixed for 3-6 hr. The tissue was subsequently postfixed in 1% osmium tetroxide for 2 hr. This recipe was used for the accessory flexor muscle of the walking legs of the crab Cancer magister and the scutal depressor muscle of the barnacle Balanus cariosus.

2). After Huddart and Oates (1970), the tissue was prefixed in 5% glutaraldehyde, 0.1 M sodium cacodylate adjusted to pH 7. After 5 hr prefixation, the tissue was washed in cacodylate buffer then postfixed in 2% of osmium tetroxide for 2 hr. This fixative was used for the prothoracic flexor tibialis muscle of the stick insect carausius morosus and for the metathoracic extensor tibialis of the locust Locusta migratoria migratorioides.

The former fixative had good penetration for flexor tibialis of E. calcarata but the tissue showed evidence of shrinkage. Calculations revealed that it was hypertonic. The latter fixative was good for peripheral fixation but penetration was poor presumably due to the lack of formaldehyde and large size of the fibres. So following Osborne's (1980) methods for the calculation of fixative osmolarity for the fixation of insect tissues, I have developed a prefixative, postfixative and buffer as shown below, which are isotonic with muscle tissue of E. calcarata and have good penetration because of formaldehyde.

Prefixative

Glutaraldehyde	0.25	M
Formaldehyde	0.1	M
Sodium cacodylate	0.05	M
$CaCl_2$	0.003	M
total	0.403	$M.l^{-1}$

Postfixative

Sodium cacodylate	0.05	M
Sucrose	0.347	M
$CaCl_2$	0.003	M
Osmium tetroxide 1%	ignored	
total	0.400	$M.l^{-1}$

Buffer		
Sodium cacodylate	0.05	M
Sucrose	0.347	M
$CaCl_2$	0.003	M
total	0.400	$M.l^{-1}$

2.1.4. Continuation of fixation

All solutions were adjusted to pH 7.4, with NaOH and the fixatives were made fresh. Fibres were prefixed for 2 hr including dissection and for the last hour of prefixation the fibres were kept in refrigerator. The fibres were washed in buffer solution, postfixed for 1 hr 30 min, then washed in buffer prior to dehydration. The tissue was dehydrated in an ethanol series and held overnight in 70% ethanol. Next day when dehydration was completed the alcohol was replaced with 50 : 50 ethanol : propylene oxide for 30 min, then placed in propylene oxide for 60 min, and subsequently transferred to 50 : 50 Emscope resin : propylene oxide and left overnight in the refrigerator. The following day the tissue was placed in Emscope resin and rotated in oven of 37°C for 4 hr, finally tissues were embedded in fresh resin overnight at 57° C.

2.1.5. Section cutting and staining

4 blocks of fibre bundles for each muscle part were chosen to cut in longitudinal and cross section for the electron and light microscope. The LKB Pyramitome and Ultratome (Reichert O M U3) were used for cutting thick and thin sections

respectively. For electron microscopy, 90 nm sections (light gold) were cut and the ribbon of sections was placed on the mat side of a grid of 300 mesh. 6 grids were prepared from each block. Grids were stained with uranyl acetate for 30 min, then were washed with distilled water, followed by lead citrate staining for 25 min. They were then washed with 0.02 M of NaOH and finally were washed with distilled water. 1 μm Sections were cut in longitudinal and cross section. These were stained with 1% toluidine blue for light microscopy to measure sarcomere length and cross sectional area of the muscle fibres.

2.1.6. Electron microscopy

The tissue was observed on the Corinth (275, AEI) electron microscope. From 20 fibres within each of the 12 muscle parts above, photographs were taken at X 4,000 and at X 40,000. Prints were made at X 20,000 for the analysis of number and percentage area of mitochondria, and percentage area of sarcoplasmic reticulum (SR). Prints were also made X 40,000 to assess thin and thick filaments numbers and ratios. A diffraction grating with 2160 lines per mm was used to calibrate the electron microscope magnification.

2.1.7. Measurements and calculations

In order to analyse the number of mitochondria per μm^2, percentage area of mitochondria and percentage area of SR of the muscle fibres, a point-counting grid with the points arranged in a triangular pattern and 1008 points on an area

of 208 cm^2 (13 cm x 16 cm) was used. A triangular grid with a greater points per unit area was chosen in preference to others as it is more accurate (Steer, 1981; Aherne & dunnill, 1982). The area of sample of the muscle was calculated, 52 μm^2.

2.1.7.1. Number of mitochondria

The test grid was randomly laid on photographs, and profiles of mitochondria located under the grid, were counted directly, then number of mitochondria of each sample was divided by the area of the sample on the muscle, 52 μm^2, to calculate the mitochondrial number per μm^2 for each muscle sample. Finally mean mitochondria number per μm^2 of all samples from each part, and from the whole muscle was calculated.

2.1.7.2. Calculation of mitochondrial area

The test grid was laid randomly on the photographs and points of the grid falling on mitochondrial profiles were counted. Then the number of points laying on mitochondria, were multiplied by the test grid area of the muscle, 52 μm^2, and divided by the total number of points of the grid, to calculate mitochondria area per sample. Finally the mean percentage area of mitochondria of fibres for each parts and for the whole muscle was calculated.

2.1.7.3. Average area of each mitochondrial profile

The area of total mitochondrial profiles per μm^2 of fibres from each individual part was divided by the number of

mitochondrial profiles per μm^2 from the each part, to calculate average area of mitochondrial profile within the individual muscle part. The same procedure was followed, to calculate the average area of mitochondrial profile for the whole muscle.

2.1.7.4. Sarcoplasmic reticulum area

The percentage area of the sarcoplasmic reticulum was calculated in the same way as for mitochondria.

2.1.7.5. Calculation of thin and thick filament density and thin to thick filaments ratio

20 samples of 64 mm^2 i.e: 8 mm x 8 mm were selected, from separate contact photographs taken from fibres within each part of the muscle. A light microscope with a square grid graticule was employed. The mean thin and thick filament numbers per μm^2 and ratio of thin to thick filaments of fibres within each part of the muscle were calculated as follows. Thin and thick filaments of each sample were counted. The number of thin filaments of each sample was divided by the number of thick filaments of the sample, to calculate thin to thick filaments ratio of each sample. The sample area of the muscle was computed, 0.04 μm^2. The number of thin and thick filaments were divided by the sample area of the muscle, 0.04 μm^2, in order to calculate the number of thin and thick filaments per μm^2 of the muscle for each sample respectively. Finally the mean and standard deviation of thin and thick filaments number per μm^2 and ratio of thin to thick filaments

of 20 samples of each muscle part and those of the whole muscle was determined.

2.1.7.6. Measuring sarcomere length

The length of 1000 sarcomeres from different blocks for each part were measured. Each time 10 consecutive sarcomeres were selected randomly and their lengths measured, using the Light microscope with an eye-piece graticule. Then the mean and STD of sarcomere lengths of fibres from each part of the muscle and the whole of the muscle, were calculated.

2.1.7.7. Measuring cross sectional area of muscle fibres

The profiles of all muscle fibres in semi thin sections were drawn on graph paper, using a microscope (leitz, SM-LUX), with drawing attachment. A calibration scale was drawn at the bottom of each graph paper, using a graded slide, with each unit of 10 μm. The number of small squares of the graph paper within each profile, was multiplied by the calibrated area of a small square, to calculate the cross sectional area of the fibre. Finally the mean and STD of fibre cross sectional areas within each part of the muscle and within the whole muscle were computed.

All sections for both electron and light microscopes were cut at different levels of fibre bundles to present sections of most parts of fibres.

All electron microscope magnification were adjusted, after

calibration of the microscope.

All measurements were tested statistically by analysis of variance (ANOVA) and when differences in populations were indicated, T-test was performed to determine which populations were different and the degree of significance.

2.2. HISTOCHEMICAL EXPERIMENTS

2.2.1. Dissection and cutting section

The methods for cutting sections for ATP and NADH-TR staining were modified from the method of Stokes et al, (1979). Following the dissection procedure above the femur was isolated, the ventral and the dorsal cuticle were removed by cutting with an abrasive disc. Two pieces of polystyrene were placed between the anterior and the posterior cuticle, to separate the cuticles and hold the fibres perpendicular to the apodeme. The muscle was washed with some drops of saline, to fill air place between the muscle fibre bundles and make it easy to cut.

The femur containing the flexor muscle, was immersed in isopentane supercooled to -160° C by liquid nitrogen, for some 10 min to freeze the muscle. The frozen muscle was transferred to the cryostat, and left for 1 hr to allow the temperature to rise to -20° C before further dissection, so that I could touch it. For each pair of metathoracic legs, the anterior cuticle was removed from one femur and the posterior cuticle

from the another, in order to cut the sections from the anterior and posterior respectively. The muscle side with the cuticle was mounted on top of a chuck, using the O.C.T compound, then the chuck was immersed in the supercooled isopentane, to make the O.C.T compound hard. The chuck with the muscle mounted on the top was kept in the cryostat for 1 hr. Sections 22 μm thick, were cut from the anterior and the posterior surfaces of the muscle, using the cryostat. The sections were picked up on clean and dry slides, and air dried for 30-40 min at room temperature before staining.

The stages above were the same for the demonstration of activity of both ATPase and the NADH-TR.

2.2.2. ATP staining

2.2.2.1. Methods of ATP staining

The method of ATP staining was modified from the method of Stokes et al, (1979). After the muscle was stained, at various temperatures and for different incubation, dehydration and clearing times, finally the recipe below was developed. The sections were pre-incubated in barbital buffer (pH 10.1), at 36°C, for 12 min. The sections were then medium incubated in ATP-barbital buffer (pH 10.1), at the 36°C, for 30 or 45 min. After that the sections were transferred to the $CoCl_2$ solution of 0.02 M, for 3 min at room temperature, then washed in 3 changes of distilled water (each time 1 min). The section then were exposed to 1% ammonium sulphide solution for 8 min. The

sections were washed for 5 min in running tap water, dehydrated with graded methanol (80%, 4 min; 90%, 5 min; 100%, 5 min) and finally cleared in Histoclear for 3 min and mounted, using Histomount (Histological Mounting Media). The incubation was done at 30 and 45 min, to produce light and dark staining respectively.

2.2.2.2. Material for ATP staining

The recipes for incubation mediums were modified from the recipe of Ogonowski and Lang (1979) following the recommendation of Padykula and Herman (1955). After several experiments at different pH and ATP concentration, the following recipes of pre and medium-incubation were selected.

Pre-incubation

1- Sodium barbital	1.03	g
2- $CaCl_2$	0.50	g
3- D.W added to	250.00	mls
4- pH	10.10	

Incubation medium

1- Barbital buffer	23.75	ml
2- 0.1 M $MgCl_2$	0.375	ml
3- ATP	0.08	g
4- pH	10.10	

Crystalline sodium ATP salt was added and final concentration of the ATP was 0.0058 M.

2.2.3. NADH-TR staining

2.2.3.1. Methods of NADH-TR staining

The method was modified from those of Stokes et al (1979) and Ogonowski and Lang (1979). After staining at several temperatures and altering incubation, dehydration and clearing times, finally the method below for the NADH staining was developed. Sections were pre-incubated in the phosphate buffer for 12 min at the temperature of 36°C. The sections were then incubated in the NADH-phosphate buffer for 2.5 hr at the temperature of 36°C. After that the sections were washed with D.W for 1 min, dehydrated with graded methanol; 90% for 1 min and 100% 2 min, cleared in Histoclear for 1 min and mounted, using Histomount.

2.2.3.2. Materials of NADH-TR staining

After testing several pH and different concentration of the substrates, the following recipe was developed. The recipe for the incubation medium was modified from those of, Ogonowski and Lang (1979) and Zimmermann and Pearse (1959). Pre-incubation was performed in the phosphate buffer.

Phosphate buffer

19 mls of the 0.1 M solution of monobasic sodium phosphate was added to 81 mls of 0.1 M solution of dibasic sodium phosphate in order to make up 100 mls of the phosphate buffer. The buffer was adjusted at pH 7.4.

Incubation medium

1-Phosphate buffer	7	ml
2-Nitro Blue Tetrazolium	10	mg
3-NADH	10	mg
4-D.W	6	ml

The 10 mg of Nitro Blue Tetrazolium (NBT) was added to the 7 ml of the phosphate buffer (solution A). The 6 ml of D.W was poured in a tube contained of 10 mg NADH (solution B). Finally the solution A and B were mixed proportionally.

2.3. PHYSIOLOGICAL EXPERIMENTS

2.3.1. Speed of the muscle contraction.

5 adult males and 5 females were used. The animals were cooled for 5 min, to immobilize them, then secured ventral side upwards on a platform (Fig, 1). A thread from one side of the lever of the isotonic transducer was attached just distal to the main tibial spine and the leg was aligned so that the movement of the tibia was in the plane of the transducer lever (Fig. 2). The other side of the lever was attached by a thread to a load via a pulley system. The load and the tibia were attached to the lever, at equal distances from the fulcrum.

The transducer was connected to a Washington pen recorder. The load was increased in stages. For every experiment the femur-tibia joint angle was set at 90 degrees. i.e. the tibia was vertical to the femur. A metal rod was used to prevent

Fig. 1: Showing experimental platform for E. calcarata. The animal was placed ventral side upwards. Each leg was secured by tying them to pillar 2, 4 and 6. To prevent animal body movement elastic bands were stretched over the thorax and abdomen to pillar 1, 3 and 5. pillar number 7 was used for secure the platform.

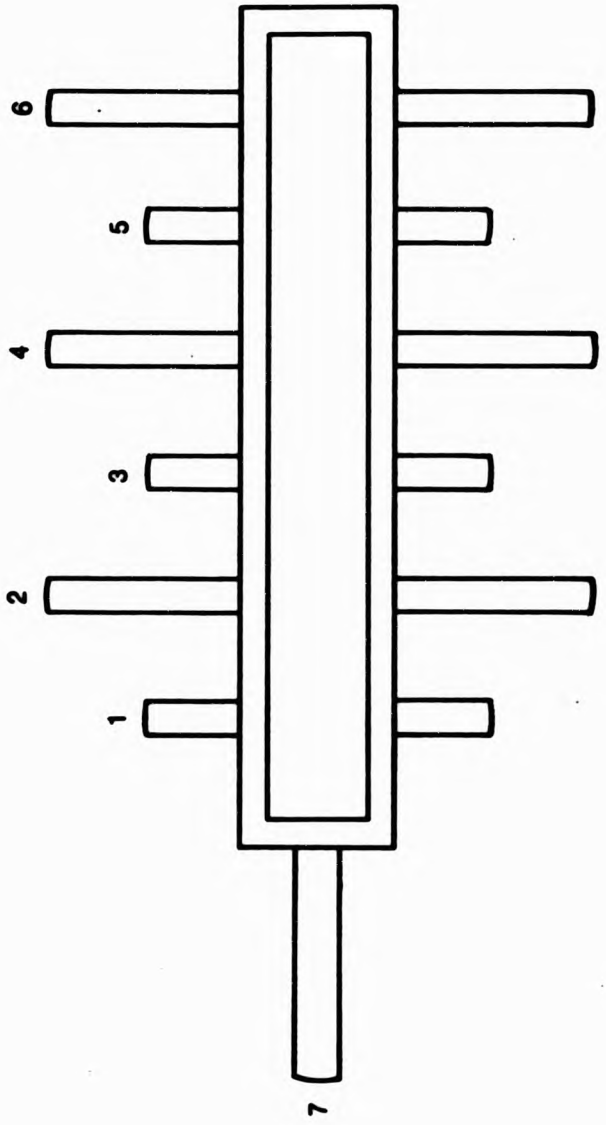
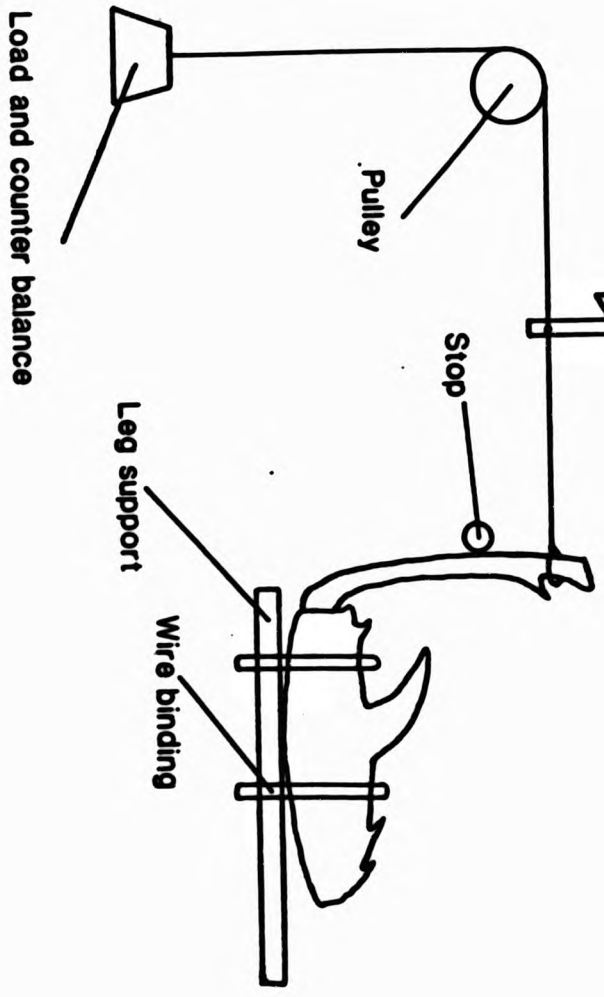

6
5
4
3
2
1
7

Fig. 2: Showing how the leg was set up on the platform, the attachments of the tibia and load to the transducer and also the counter balance. The leg was aligned so that the movement of the tibia was in the plane of the transducer lever movement.


Transducer
Pulley
Stop
Leg support
Wire binding
Load and counter balance

extension of the tibia by the load to angles greater than 90 degrees (Fig. 2), and the pen recorder was adjusted to a base line on the chart recorder paper. The washington attenuator was set to a calibrated position which ensured that whole traces could be drawn on the chart recorder paper by the pen recorder. The animal was then stimulated with single 50 V pulses, using an electrical stimulator (Tektronix). The stimulus was applied across the thorax by saline pads applied to the prothoracic femur-tibia-articulations. The output from the Washington pen-recorder was fed to a frequency modulated tape recorder, so that the traces drawn by the pen recorder were also recorded on tape. The traces were ultimately displayed on an oscilloscope screen, so that the velocity of the movement could be measured more accurately.

Five experiments were carried out on each individual animal. The mean and STD for the velocity of the muscle against increasing load, was calculated for the 5 tests on an individual animal and for all tests (N=25) from all animals. A single 50 V pulse stimulus was used as this voltage was strong enough to produce a maximal response, while the single stimulus did not injure the animal.

2.3.2. Work done by the muscle

Using the traces from the Washington pen recorder the mean and STD of the initial and secondary work done was calculated in g.mm, for 5 tests on an individual animal and for all the tests (N=25). The height of initial and secondary movements

were measured from the Washington pen recorder traces which was set at speed of 1 $mm.s^{-1}$. Also the traces were transferred from the tape recorder to the pen recorder which was run at a speed of 25 $mm.s^{-1}$, to ensure that the height of initial and secondary movements were accurately measured.

2.3.3. Force produced by the initial contraction of the muscle

The mean and STD of force produced by the initial contraction of the muscle was calculated, in terms of mN, for 5 tests on an individual animal and for all tests (N=25). The force produced was calculated by the equation;

$$F=Kg.m.s^{-2}$$

Where F is the force in newtons produced by the muscle, Kg is a load in Kilogram that the muscle sustained during contraction, m is the distance which the muscle contracts in metres and s is the duration in seconds of the contraction of m meters.

2.3.4. Tension produced by the muscle at maximum load

The tension produced per cm^2 and per gram weight of the muscle was directly calculated, when the muscle sustained maximum load. The maximum load in gram sustained by the muscle was divided by cross sectional area of the muscle in order to calculate the tension produced per cm^2 of the muscle and the maximum load divided by the weight of the muscle to calculate the tension produced per gram of the muscle weight.

2.3.5. Work output and power output of the muscle.

Work output and power output of the muscle were calculated in terms of $J.Kg^{-1}$ and $W.Kg^{-1}$ of the muscle weight, when the muscle sustained different loads, respectively. The equations $J=Kg.m^2.s^{-2}$ and $W=Kg.m^2.s^{-3}$ were used in order to calculate the work output and power output produced by the muscle respectively. Where the J is work output in Joules, W is power output in Watts, Kg is the load sustained by the muscle, m is the distance in meters which the muscle contracted and the s in seconds is duration of contraction.

In order to relate the movement of the tibia to the physiological properties of the muscle, the tibial moment arm and the flexor muscle moment arms were measured. Correlation and regression analysis was carried out to compute the muscle movements from the movements of the tibia. The tibial lever factor for the male and the female are 6.96:1 and 10.18:1 respectively. The lever movement of the transducer was calibrated using a micrometer gauge.

2.3.6. Determination of the femur-tibia joint angle at which the muscle produced most tension.

6 Males were used, and on each animal 5 experiments were performed. The mean and STD of the tension produced at each angle for 5 tests on an individual animal, and for all tests (N=30) was measured. The Washington pen recorder and an isometric transducer were used. The animal was placed on the platform and stimulated with single pulses of 50 V. The angle

was decreased in steps of 0.175 rad, 10 degree, from totally extended the tibia, at 2.45 rad to full flexion of the tibia. A metal template bar was used to set each angle. The transducer was counter balanced, since the force produced by some muscles was too high for the transducer, to do this a load was attached to the transducer (Fig. 2). The load was not constant for all animals and angles, because at some angles the animal produced much higher force than at others. Arc-sin transformation was performed on the data to allow calculation of mean percentage tension.

All physiological experiments on the muscle were performed at 21°C-23°C, the temperature at which the insects were cultured.

2.4. DIMENSION AND MECHANICAL PROPERTIES OF THE METATHORACIC LEG AND THE MUSCLE

2.4.1. Dimensions of the leg

The dimensions of the metathoracic leg of the male were measured from photographs. 20 totally extended legs were photographed with a scale, and all measurements made from the photographs. The weight of the isolated legs were measured.

2.4.2. Weight and cross sectional area of the muscle

The mass of the muscle was measured in grams as below. First the dorsal cuticle of the femur was cut and all soft tissue, such as; extensor and accessory muscles, nerves, trachea were removed. Second the femur with the flexor muscle was fixed for

some 15 min in the fixation, as mentioned above for dissection of the muscle fibres. Finally the muscle was dissected out whole with apodeme, dried in a desiccator to constant weight and weighed.

The cross sectional area of the muscle was calculated as follows: 1) The volume of the muscle was measured by immersing the muscle in a 10 ml graduated glass tube of saline. The volume of saline displaced in ml was the muscle volume. 2) Several muscle fibre bundles were dissected from the apodeme and internal walls of the femur at different parts of the muscle and the fibre lengths measured under a microscope equipped with a eye-piece graticule. Then all muscle fibres were dissected from the apodeme and the weight and volume of the dried apodeme were measured. The weight of the apodeme was deducted from the total weight of the muscle and apodeme, to calculate the muscle weight. The volume of the apodeme was deducted from the volume of the muscle and apodeme to measure the volume of the muscle. After the weight and volume of the muscle were measured, the density, Q, of the muscle was measured using the equation,

$$Q = \frac{m_m}{v_m}$$

Where m_m is the muscle weight in gram and v_m is muscle volume in ml. Finally the cross sectional area of the muscle was calculated using the equation below from Gray and Mill (1983).

$$a_m = \frac{m_m}{l_m} \times Q$$

Where l_m is the mean muscle fibre lengths in cm and Q is the density in $g.mm^{-3}$.

The mean and STD of the weight and cross sectional area of the muscle was calculated for 20 muscles.

2.4.3. Measuring femur-tibia-angle in different positions

From the each of 20 metathoracic legs 3 photographs were taken at different femur-tibia-angles i.e: with the fully extended tibia, with the tibia just touching the top of the main spine of the femur and with the tibia fully flexed. Finally the mean joint angle at each position was calculated.

2.4.4. Data analysis of mechanical properties of the leg and the muscle

The moment arm of the flexor tibialis muscle acting about the femur-tibia articulation was measured directly to an accuracy of 0.01 mm, at various flexion angles of the femur-tibia joint, covering the range of possible movements of the tibia in steps of 0.35 rad (Fig. 62, upper diagram).

To measure the angular velocity of tibial movement at each flexion angle, the distance of muscle shortening for each angular movement of the tibia in steps of 0.35 rad was calculated, using the scale diagram shown in fig. 3. AB is the muscle moment arm of the totally extended tibia at 2.45 rad, AC is the moment arm at a flexion angle of 2.10 rad and so on. B,C,D...K are the active attachment points of the muscle with

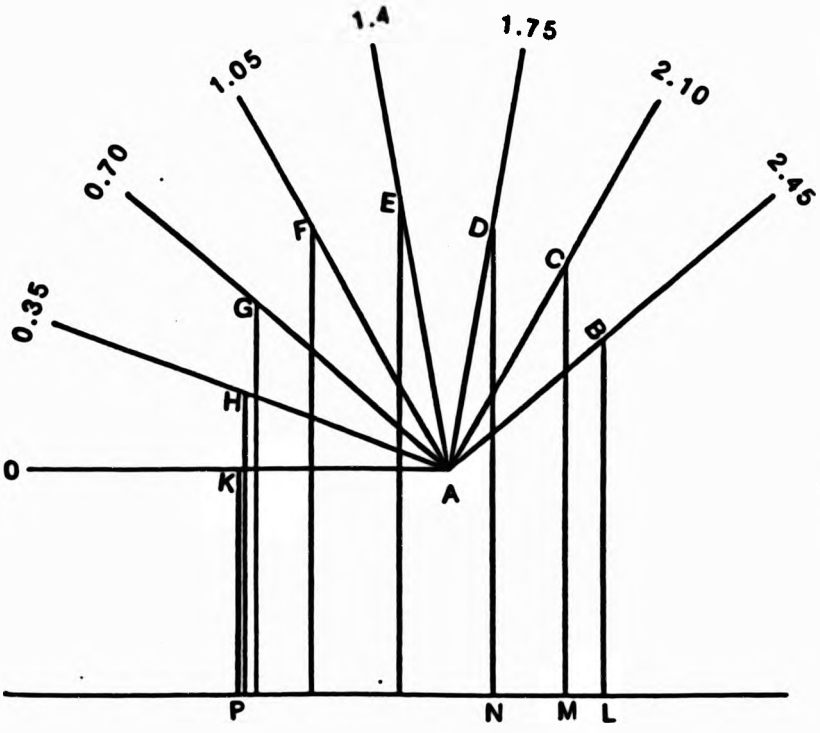


Fig. 3: The scale diagram showing shortening distance of the flexor muscle, throughout angular movement of the tibia in 0.35 rad steps.

the tibia at different angles and A is the femur-tibia articulation. LM is the shortening distance of the muscle when the tibia moves 0.35 rad, from 2.45 to 2.10 rad and the LN is the distance when the tibia moves 0.70 rad, from 2.45 to 1.75...LP is when the tibia moves from full extension to full flexion. Figure 9 shows the attachment of the flexor and extensor muscle apodemes relative to the condyles in the femur.

Knowing the distance of shortening of the muscle for each angular movement of the tibia and the maximum muscle velocity, assuming that the muscle velocity is constant through the tibial movement, the time of angular movement of the tibia for each displacement was calculated by equation,

$$dt=dx/V$$

Where dt in seconds is the time of muscle shortening of distance dx in mm, and V is muscle velocity in $mm.s^{-1}$. The time was measured for each angular movement of the tibia throughout the flexion.

Then angular velocity of the tibia at each flexion angle was estimated from a plot of the angular displacement (rad) of the tibia against time (s) of each angular movements during the strike. In order to calculate the angular velocity at each point of tibial movement, the slopes before and after each points were measured and the mean slope was calculated.

To estimate angular acceleration of the tibia, a plot of the

angular velocity against time for the angular movement of the tibia was drawn. To calculate the angular acceleration at each point of tibial movement the slopes before and after of each point were measured and the mean slope was calculated. The mean and S.E.M. of the angular velocity and angular acceleration of the tibia were measured for each tibial movements in steps of 0.35 rad, throughout the defensive strike, from 10 measurement of the moment arms.

3. RESULTS

3.1. ANATOMY OF THE METATHORACIC LEG AND THE FLEXOR TIBIALIS MUSCLE

The morphology of the metathoracic leg and the flexor tibialis muscle was studied to find the correlation between the morphology of the leg, the muscle and their functions.

Table 1 shows the size and weight of the intact male and the female, the dimensions and weight of the metathoracic leg and the flexor tibialis muscle of the male and the female E. calcarata and fig. 4 shows the dimensions of the leg which were measured. The most remarkable morphological difference between the male and the female is between their metathoracic legs (fig. 5 and 6). The metathoracic leg of the male E. calcarata is highly specialized to its behavioural function, the defensive strike, with its large metathoracic femur, length 32.7±0.45 mm and depth 9.4±0.27 mm. The femur is armed with 4 spines of which the third one from the proximal end is very large, 6.2±0.11 mm (Fig. 7). Whereas the femur of the female is much smaller, length 28.98 mm and depth 6.11±0.19 mm, the main femur spine of the female is also much smaller, 2.14±0.06 mm (Fig. 8). The wall of the femur is made of thick cuticle to cope with high force generated by the flexor tibialis muscle and is not easily broken when the insect catches a strong object. The long tibia of the male, 27.2±0.31 mm, bears several spines distally and the proximal part of the

Table 1: showing the mean and S.E.M (N=20) of dimensions and weight of the metathoracic leg, the weight and cross sectional area of the muscle, the size and weight of of the male and the female.

	Male	Female
1-Length of tibia (mm)	27.2±0.31	28.35±0.42
2-weight of tibia (g)	0.19±0.01	0.12±0.01
3-Length of femur (mm)	32.7±0.45	27.89±0.40
4-Depth of femur (mm)	9.4±0.27	6.11±0.19
5-Width of femur (mm)	8.3±0.20	4.84±0.12
6-Height of main femoral spine (mm)	6.2±0.11	2.14±0.06
7-Distance from articulation to main tibial spine (mm)	20.3±0.25	NA
8-Angle of total flexion tibia (rad)	0.22±0.02	0.17±0.02
9-Opening angle of total extension tibia (rad)	2.45±0.03	2.62±0.03
10-Opening angle at which tibia reaches to top of femoral spine (rad)	0.28±0.02	NA
11-Weight of the leg (g)	1.44±0.09	0.61±0.04
12-Weight of the tibialis flexor muscle (g)	0.466±0.02	0.247±0.02
13-Cross sectional area of the muscle (cm^2)	1.81±0.07	1.07±0.08
14-Size of the animal form the top of head to the end of tail (cm)	11.17±0.2	13.20±0.31
15-Weight of the intact animal (g)	14.42±0.34	23.71±0.42

Fig 4: Shows dimensions of the metathoracic leg of the male E. calcarata. L1, length of the femur; L2, length of the tibia; L3, distance from tip of the main spine of the tibia to the femur-tibia articulation; H1, height of the main spine of femur; H2, depth of the femur from ventral surface to the dorsal surface (excluding spines); a, The femur-tibia joint angle.

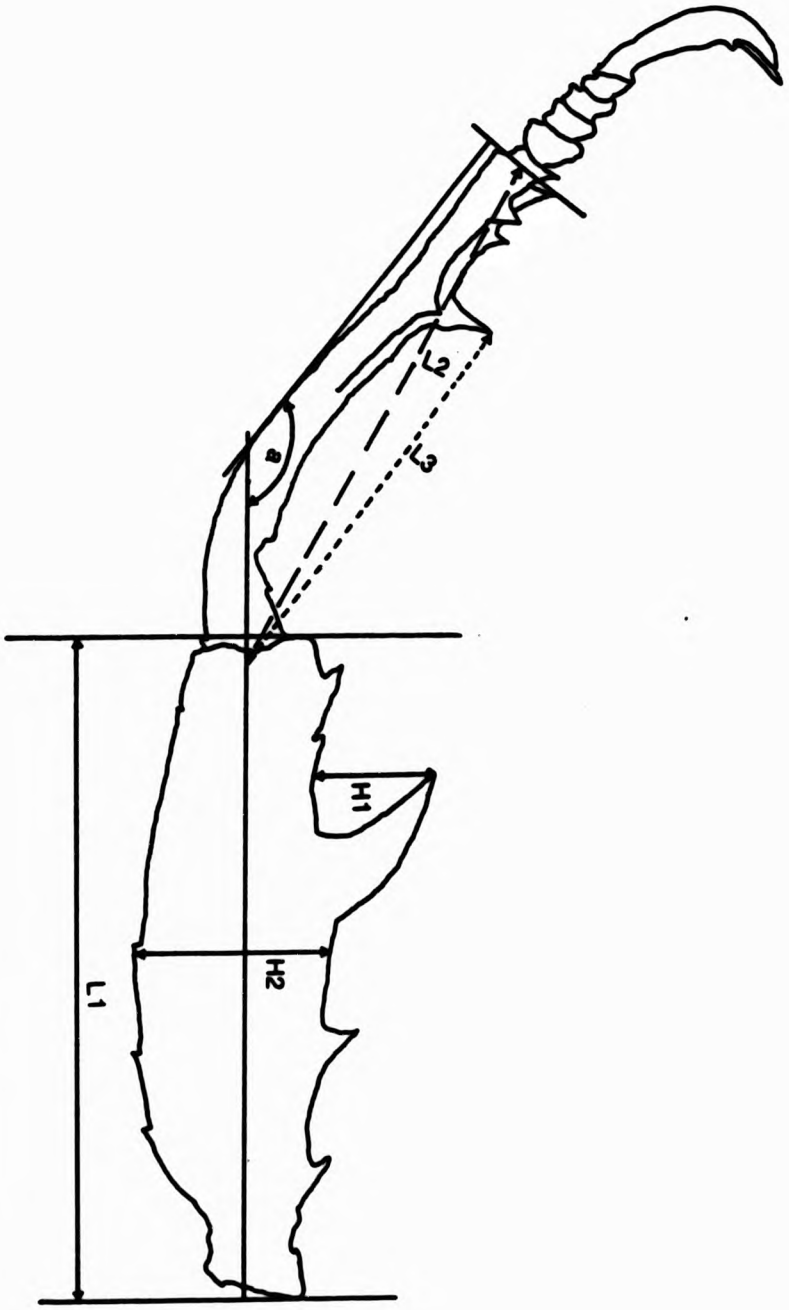

L2
L3
θ
H1
H2
L1

Fig 5: The male E. calcarata in defensive posture. The end of it,s abdomen bent upwards. X 1.1

Fig. 6: The female E. calcarata in defensive posture. The abdomen flexed upwards with the abdomen tip flexed downwards. X 1

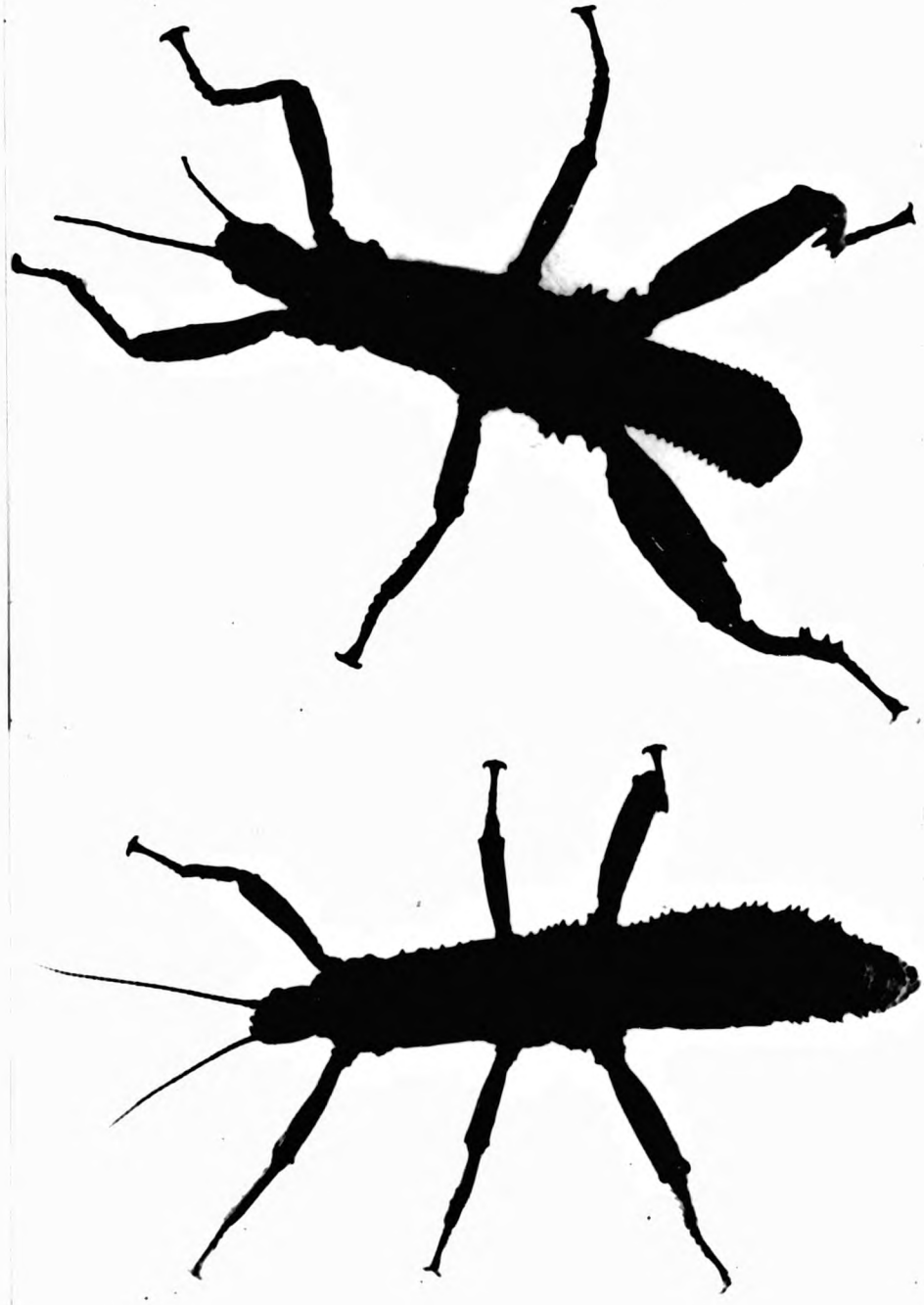


Fig. 7: The metathoracic leg of the male E. calcarata.
X 2.5

Fig. 8: The metathoracic leg of the female E. calcarata.
X 2.5

tibia, between the largest spine and the femur-tibia articulation has length of 20.3±0.25 mm. The proximal tibia is curved outwards which may enable the insect to grasp larger objects between the main femur and tibial spines (fig. 4). The main femur spine located on ventral surface of the femur so that when the tibia is fully flexed the tibia does not touch the spine but passes close to the spine on the anterior side of the spine. The tibia of the female is slightly larger, 28.35 mm, however the tibia of the female is straight from distal at the proximal end (Fig. 7 and 8).

The tibia is moved by two muscles. The extensor tibialis muscle is a pinnate muscle attached between a long central apodeme and the dorsal wall of the femur. The distal end of apodeme for the extensor muscle is attached to the tibia, dorsal to the paired condyles (Fig. 9). The flexor tibialis muscle is much larger than the extensor tibialis muscle and fills most of the space within the femur. The flexor muscle is a pinnate muscle, attached to a large central apodeme and to the anterior and posterior walls of the femur (Fig. 10). The flexor tibialis apodeme attaches to the proximal end of the tibia at the femur-tibia articulation (Fig. 9). The condyles of the femur-tibia articulation are located between the flexor and extensor muscles attachments to the tibia. The flexor muscle of the male, with a weight of 0.466±0.02 g and cross sectional area of 1.81±0.07 cm^2, is much larger than that of the female, with weight of 0.247±0.02 g and cross sectional area of 1.07±0.0.08 cm^2. In the male E. calcarata

Fig. 9: Shows the attachment of the flexor and extensor muscle apodemes relative to the femoral condyles about which the tibia rotates.

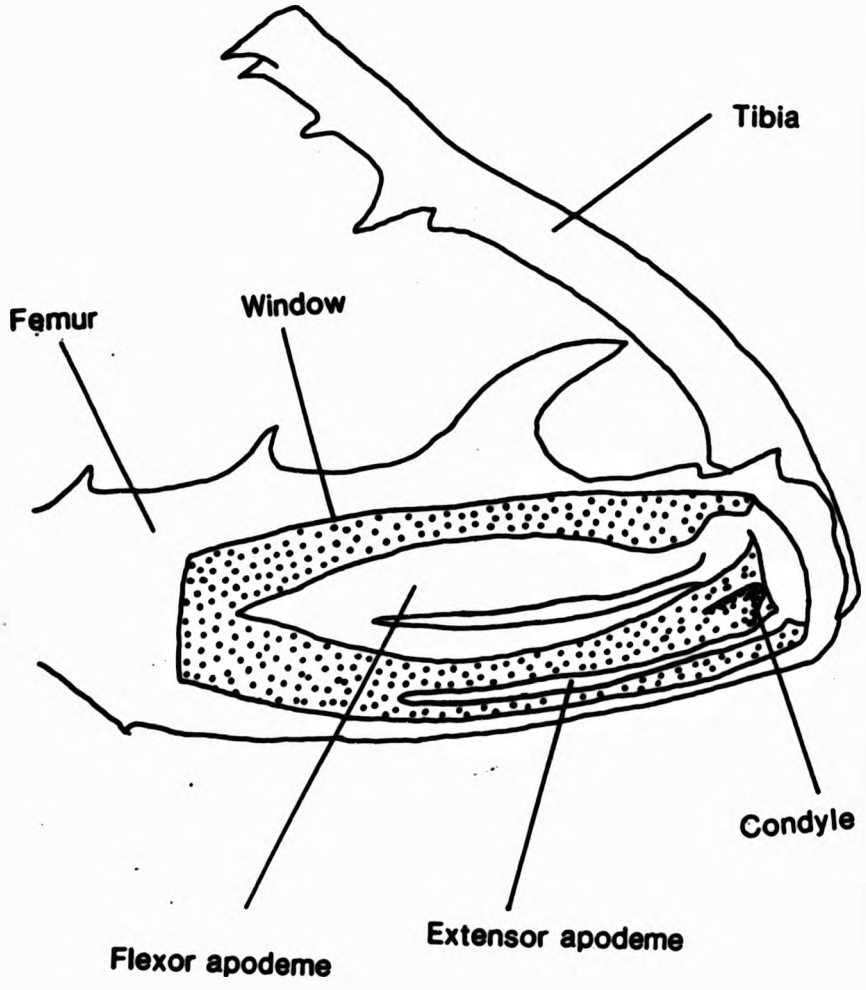
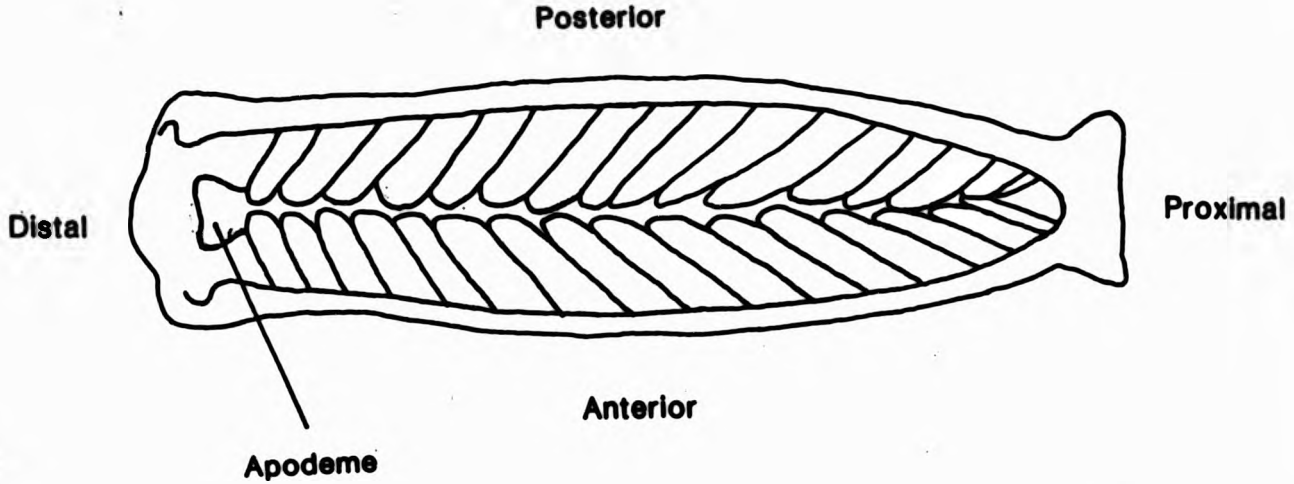

Tibia
Femur
Window
Condyle
Extensor apodeme
Flexor apodeme

Fig. 10: The arrangement of the fibres of the flexor tibialis muscle and the attachment of the fibres to the anterior and posterior cuticle of the femur and to the central apodeme.


Posterior
Distal
Proximal
Anterior
Apodeme

the defensive strike is performed by the metathoracic flexor tibialis which is the principal and the most forceful muscle.

3. 2. HISTOCHEMICAL PROPERTIES OF THE MUSCLE

3. 2. 1. ATPase activity

Fig. 11 shows the pattern of staining for myosin adenosine triphosphatase (ATPase) activity in a transverse section through the anterior head of the flexor tibialis muscle from the male. Transverse sections from the anterior and posterior heads of the muscle exhibited similar uniform patterns of staining. There were no large areas where fibres stained lighter indicating slower fibres. Closer observation revealed small regions of the muscle where fibres stained lighter (fig 13, 14, 15). These fibres represented less than 15% of the total muscle fibres. Although the lighter stained fibres were scattered through the muscle, they were more frequent in the dorsal regions. The muscle is therefore a mixture of fibres with different histochemical properties, but primarily consists of fibres which stained darker and a smaller number of fibres which stained lighter for ATPase. The fibres which stained darker tended to have larger cross sectional area (fig. 13, 14, 15).

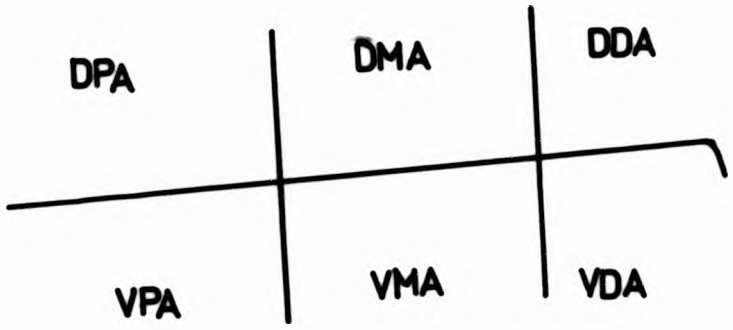
The pattern of staining for ATPase activity of the muscle from the female was similar to that of the male, however the distribution of fibre types of the female tended to be more uniform with more lighter stained fibres.

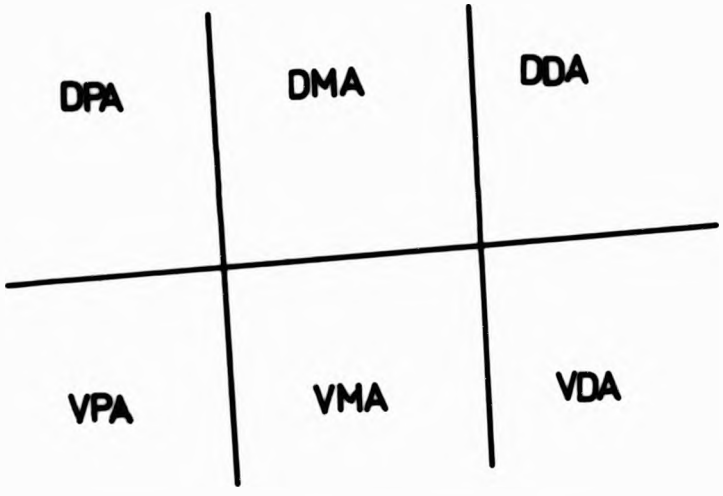
3. 2. 2. NADH-TR activity

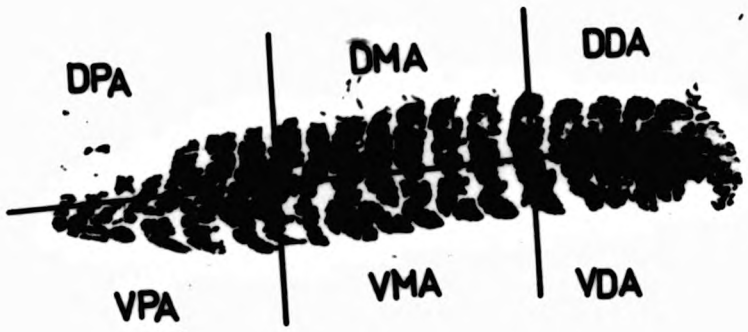
Fig. 12 shows the pattern of staining for nicotine adenine

Fig. 11: Showing the distribution of the fibres with different densities of staining for ATPase activity in a transverse section through the anterior head of the muscle, dorsal regions uppermost and proximal to the left. X 5.8.

Fig. 12: Showing the distribution of the fibres with different densities of staining for NADH-TR activity in a transverse section through the anterior head of the muscle, dorsal regions uppermost and proximal to the left. X 6.1.


DPA
DMA
DDA
VPA
VMA
VDA


DPA
DMA
DDA
VPA
VMA
VDA


DPA
DMA
DDA
VPA
VMA
VDA

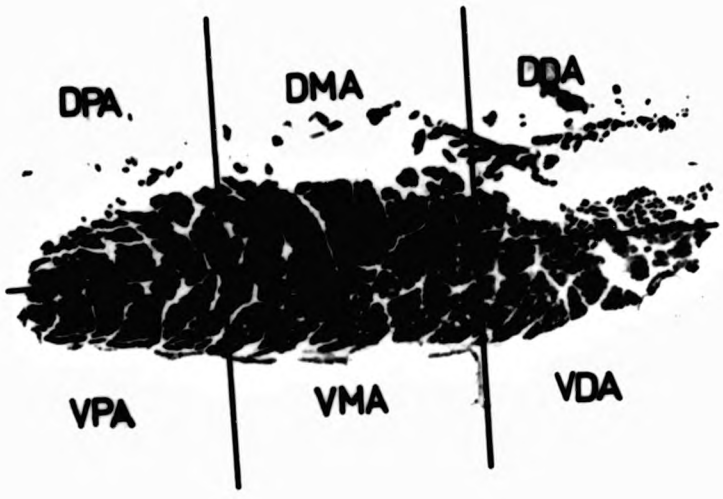

DPA
DMA
DDA
VPA
VMA
VDA

Fig. 13: A portion of transverse section of the anterior dorsal medial region of the muscle, stained for ATPase activity, showing large fibres stained darkly "fast fibres" and small fibres stained lightly "slow fibres". It also shows that in some areas there are more fibres which stain with high density than those that stained with low density. X 100.

Fig. 14: A portion of transverse section of the anterior dorsal distal region of the muscle, stained for ATPase activity, showing the large darkly stained fibres, fast fibres. X 100

Fig. 15: A portion of transverse section of the anterior ventral proximal regions of the muscle, stained for ATPase activity, showing some fibres stained extremely light "slow", some extremely dark "fast". X 100

L

S

L S

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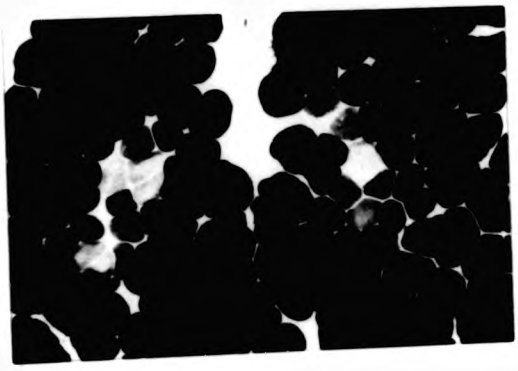

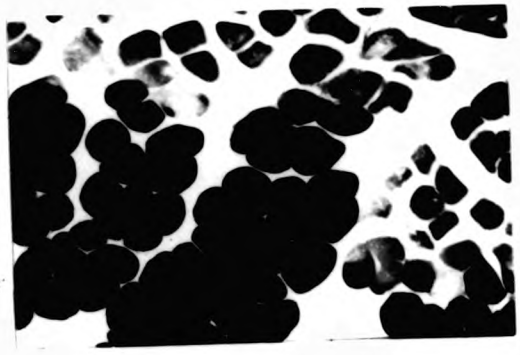

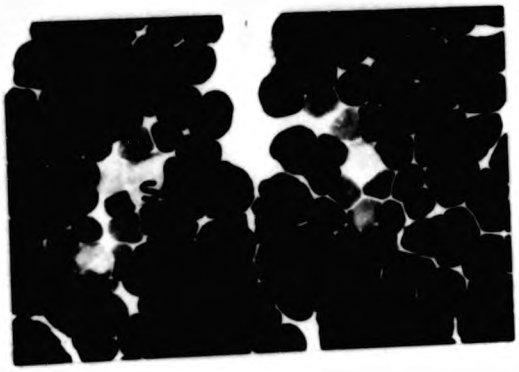

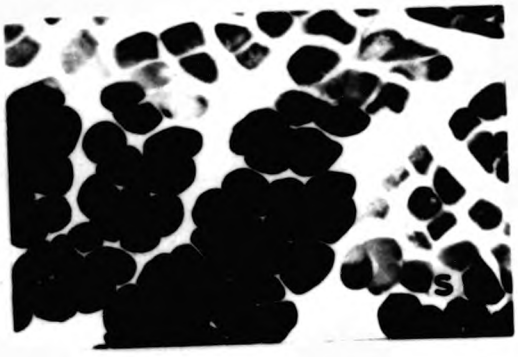

dinucleotide-tetrazolium reductase (NADH-TR) activity of the anterior transverse section of the muscle from the male. Transverse sections from the anterior and posterior heads of the muscle exhibited similar patterns of staining i.e. there were no large areas where the muscle fibres stained significantly darker, indicating slower fibres. As with ATPase staining small groups of fibres stained with a different intensity indicating the presence of slower fibres. The fibres which stained significantly darker were more frequent in dorsal regions than ventral regions, also most of these fibres correspond in location with fibres which stained lighter for ATPase (fig. 11, 12). The muscle was therefore a mixture of fibres where minority of fibres stained darker for NADH-TR, and the majority stained lighter. The muscle fibres that stained darker for NADH-TRase activity tended to have smaller cross sectional area (fig, 16, 17).

The pattern of NADH-TR activity for the muscle of the female was similar to that of the male, however the female tended to have more darker staining fibres.

Approximately 50% of the muscle fibres stained darker for ATPase activity and lighter for NADH-TR activity. In some areas fibres stained darker for both ATPase and NADH-TRase activities (approximately 35%), these were in some dorsal regions and also in some ventral parts, VPA and VPP. Moreover in some small areas fibres stained darker for NADH-TRase and lighter for ATPase activity (approximately 15%) e.g. DMA and

Fig. 16: A portion of transverse section of the anterior dorsal distal region of the muscle stained for NADH-TR activity, showing the large lightly stained fibres "fast fibres" and small darkly stained fibres "slow fibres". X 100

Fig. 17: A portion of transverse section of the anterior ventral proximal region of the muscle, stained for NADH-TR activity, showing fibres which stained with low, intermediate and high staining densities mixed randomly.
X 100

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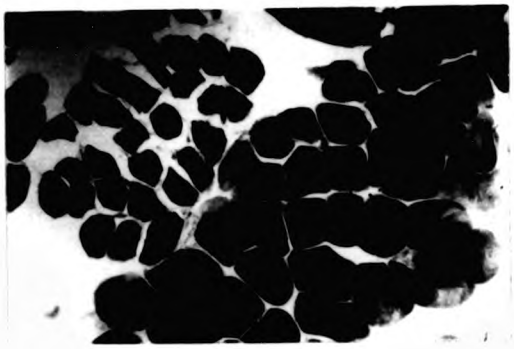
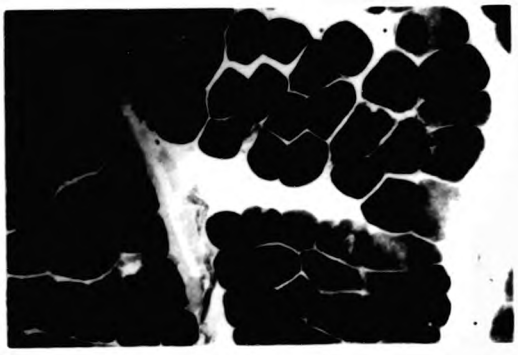
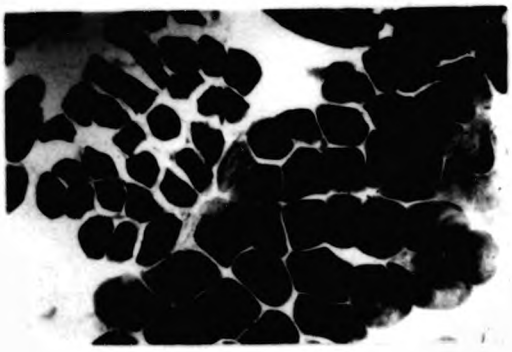
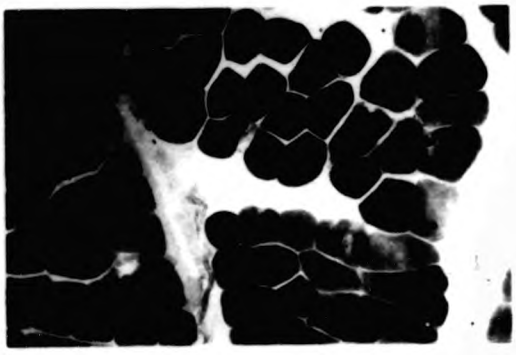








DMP (fig. 11, 12). There were extremely low numbers of fibres which stained lighter for both enzymes.

The distribution and relative intensity of the staining of the muscle fibres from the female were similar to those of the male. However there were more fibres which stained lighter for ATPase activity and darker for NADH-TR activity in the female than in the male. The intensity of both enzymes, ATPase and NADH-TR, activities for both muscles of the male and the female showed that, the muscles were a mixture of fibres with different intensity staining for ATPase and NADH-TR with primarily of fibres which stained darker for ATPase and lighter for NADH-TR.

3.3. THE MUSCLE ULTRASTRUCTURE

Sarcomere lengths were measured randomly on longitudinal sections cut at different depths throughout the muscle fibre bundles. The fibre bundles were sampled from 12 parts of the muscle from the adult males and females. Cross sections were obtained from the muscle fibre bundles of the 12 parts in order to analyse the fibres cross sectional area within the muscles.

Photographs taken from cross sections cut at different depths within the muscle fibre bundles for the 12 parts of the muscles were used, in order to analyse, 1. the ratio of thin to thick filaments, 2. the filaments number per um^2, 3. the mitochondrial area, 4. the number of mitochondria, 5. the average size of mitochondria and 6. the area of sarcoplasmic reticulum (SR) within the muscles. Sample areas were selected randomly from the photographs.

Analysis of Variance (ANOVA) was performed, in order to determine the degree of homogeneity between the 12 muscle parts within the muscles and between the whole muscle of the male and the female. The ANOVA was followed by T-tests to determine the degree of significances between different muscle parts. The analysis was performed for all of the ultrastructural subjects mentioned above.

3.3.1. Sarcomere length

The parts with the shortest and longest sarcomeres within the muscle of the male were DDA and VMP with mean sarcomere lengths of 3.97±0.93 µm and 6.30±0.40 µm respectively (table 2a). The ANOVA test for the sarcomere lengths showed that the population was not homogeneous and that some muscle parts within the male were significantly different from others ($p < 0.001$). The T-test between individual parts showed that most parts of the muscle from the male were significantly different from each other ($p=0.05$). The muscle parts ranked with regard to mean sarcomere lengths of their fibres (table 2a; fig. 18) showed that only the parts which were juxtaposed within the ranking were not significantly different from each other.

The ANOVA test showed that the population of the dorsal region of the male, with mean sarcomere lengths of 5.12±1.07 µm, was significantly different from that of the ventral region, with mean of 5.77±0.66 µm, this indicates that some of the parts of either region are different from all of the parts of the other region ($p < 0.001$). The dorsal parts tended to have short to medium length sarcomeres and ventral parts to have medium to long ones. The range of sarcomere lengths within the dorsal parts usually was greater than within the ventral parts. The distal parts tended to have short to medium sarcomeres and the medial parts medium to long sarcomeres. The mean and range of sarcomere lengths for the whole muscle from the male were 5.44±0.95 µm and 1.96-9.66 µm respectively (table 3a).

Table 2: Mean sarcomere lengths of fibres in the 12 parts of the flexor tibialis muscle of a) male and b) female ranked from the shortest to the longest. Significant difference between pairs was tested at p=0.05. (x) indicates no significant difference, (-) significant difference.

a)

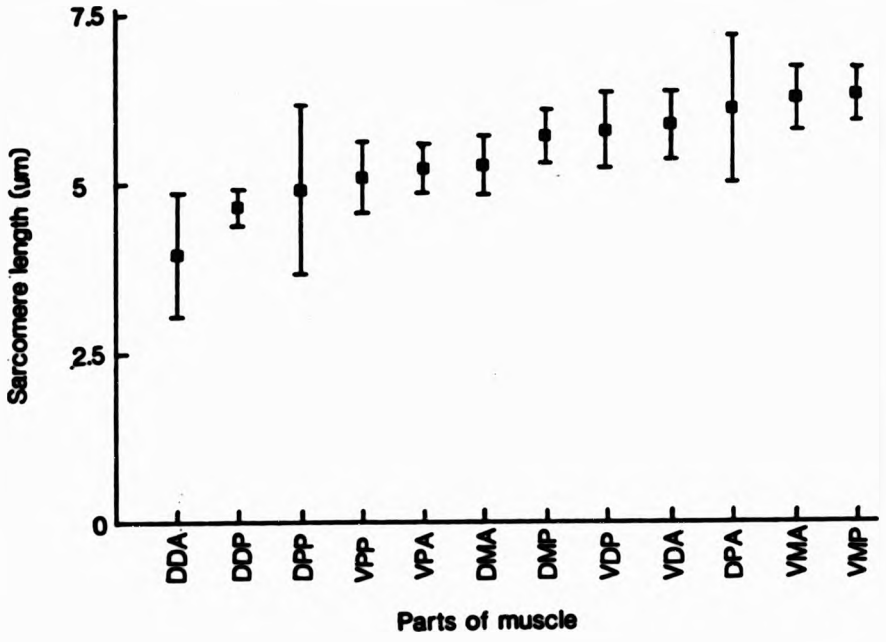
	DDA	DDP	DPP	VPP	VPA	DMA	DMP	VDP	VDA	DPA	VMA	VMP	MEAN±STD (μm)
DDA		–	–	–	–	–	–	–	–	–	–	–	3.97±0.93
DDP	–		–	–	–	–	–	–	–	–	–	–	4.67±0.27
DPP	–	–		x	–	–	–	–	–	–	–	–	4.94±1.23
VPP	–	–	x		–	–	–	–	–	–	–	–	5.11±0.52
VPA	–	–	–	–		x	–	–	–	–	–	–	5.24±0.35
DMA	–	–	–	–	x		–	–	–	–	–	–	5.29±0.43
DMP	–	–	–	–	–	–		x	–	–	–	–	5.71±0.40
VDP	–	–	–	–	–	–	x		x	–	–	–	5.80±0.56
VDA	–	–	–	–	–	–	–	x		x	–	–	5.88±0.49
DPA	–	–	–	–	–	–	–	–	x		x	x	6.11±1.08
VMA	–	–	–	–	–	–	–	–	–	x		x	6.26±0.46
VMP	–	–	–	–	–	–	–	–	–	x	x		6.30±0.40

b)

	VPA	DDA	DPA	DMA	VDP	VMA	DMP	VDA	DDP	DPP	VPP	VMP	MEAN±STD (μm)
VPA		x	–	–	–	–	–	–	–	–	–	–	4.50±0.59
DDA	x		–	–	–	–	–	–	–	–	–	–	4.65±0.69
DPA	–	–		–	–	–	–	–	–	–	–	–	4.94±0.70
DMA	–	–	–		x	–	–	–	–	–	–	–	5.10±0.31
VDP	–	–	–	x		x	–	–	–	–	–	–	5.18±0.62
VMA	–	–	–	–	x		x	x	x	x	–	–	5.37±0.76
DMP	–	–	–	–	–	x		x	x	x	–	–	5.48±0.52
VDA	–	–	–	–	–	x	x		x	x	x	x	5.52±0.64
DDP	–	–	–	–	–	x	x	x		x	x	x	5.52±1.04
DPP	–	–	–	–	–	x	x	x	x		x	x	5.55±0.61
VPP	–	–	–	–	–	–	–	x	x	x		x	5.70±0.85
VMP	–	–	–	–	–	–	–	x	x	x	x		5.70±0.69

Fig. 18: Showing; the ranked muscle parts from the male, according to their mean sarcomere lengths of fibres.

Fig. 19: Showing; the ranked muscle parts from the female, according to their mean sarcomere lengths of fibres.


7.5
5
2.5
0
Sarcomere length (μm)
DDA
DDP
DPP
VPP
VPA
DMA
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VDA
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VMA
VMP
Parts of muscle

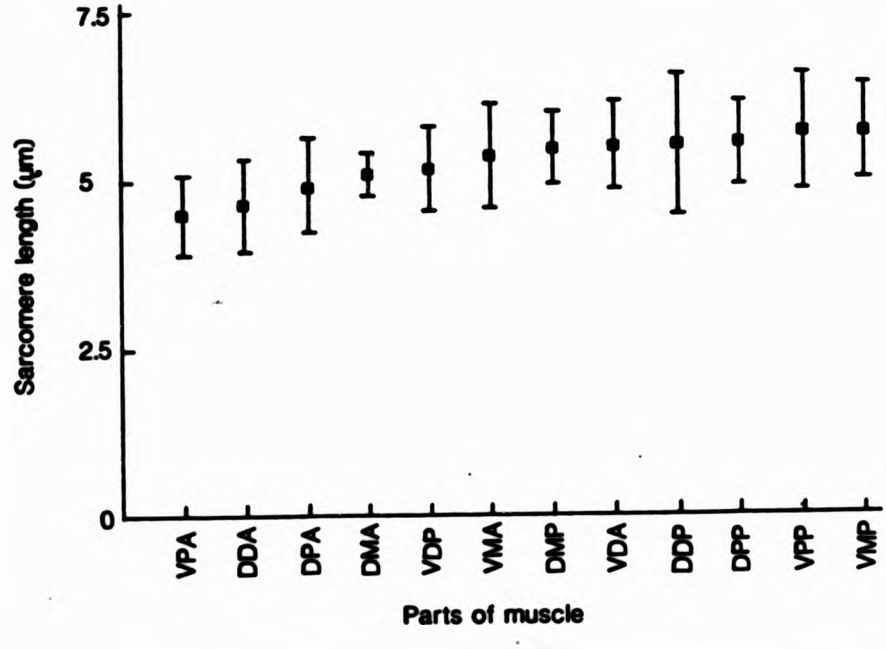

7.5
5
2.5
0
Sarcomere length (μm)
VPA
DDA
DPA
DMA
VDP
VMA
DMP
VDA
DDP
DPP
VPP
VMP
Parts of muscle

Table 3: Mean, standard deviation and range of sarcomere lengths of fibres within the 12 parts and the flexor tibialis muscle of a) male and b) female. The number in the final column shows the rank position of sarcomere lengths (mean) of the 12 muscle parts. Number (1) indicates the shortest and number (12) the longest sarcomere.

a)

MUSCLE PARTS	MEAN±STD (μm)	RANGE (μm)	RANK
DDA	3.97±0.93	1.96-5.18	1
DDP	4.67±0.27	3.64-5.32	2
DMA	5.29±0.43	4.06-6.02	6
DMP	5.71±0.40	4.48-6.86	7
DPA	6.11±1.08	4.76-9.66	10
DPP	4.94±1.24	2.38-7.84	3
VDA	5.88±0.50	3.78-7.27	9
VDP	5.80±0.56	4.34-7.14	8
VMA	6.26±0.46	5.18-9.38	11
VMP	6.31±0.40	5.46-7.28	12
VPA	5.24±0.35	4.20-6.16	5
VPP	5.11±0.52	3.92-6.58	4
WHOLE MUSCLE	5.44±0.95	1.96-9.66	

b)

MUSCLE PARTS	MEAN±STD (μm)	RANGE (μm)	RANK
DDA	4.65±0.69	2.24-5.60	2
DDP	5.52±1.04	2.38-7.70	9
DMA	5.10±0.31	4.62-7.14	4
DMP	5.48±0.52	4.52-7.05	7
DPA	4.94±0.70	2.66-7.00	3
DPP	5.55±0.61	4.34-6.86	10
VDA	5.52±0.64	2.52-7.00	8
VDP	5.18±0.62	3.22-6.72	5
VMA	5.37±0.76	3.36-6.72	6
VMP	5.70±0.69	2.80-7.00	12
VPA	4.50±0.60	2.80-5.60	1
VPP	5.70±0.85	3.08-7.14	11
WHOLE MUSCLE	5.27±0.79	2.24-7.70	

The parts with shortest and longest sarcomeres within the muscle of the female were VPA and VMP with mean sarcomere lengths of 4.50±0.59 μm and 5.70±0.69 μm respectively (table 2b). The ANOVA test for sarcomere lengths of the muscle parts of the female showed that there were significant differences between some of the parts, therefore the population was not homogeneous ($p < 0.001$). The T-test between individual parts showed that the majority of the muscle parts were significantly different from each other ($p=0.05$). The muscle parts ranked with regard to the mean sarcomere lengths of their fibres (table 2b; fig. 19) showed that, the parts with longer sarcomeres did not tend to be significantly different from each other.

The ANOVA test showed that the population of the dorsal region of the female, with mean sarcomere lengths of 5.21±0.76 μm, was significantly different from that of the ventral region, with the mean of 5.33±0.80 μm, this indicates that some of the parts of either region are different from all of the parts of the other region ($p=0.003$). The majority of parts with shorter sarcomeres were located in the dorsal region. The parts with shortest and longest sarcomeres were situated in the ventral region. The distal parts tended to have short to medium sarcomere lengths, the medial parts medium to longer and the proximal parts tended to have shorter and longer sarcomere lengths. The mean and range sarcomere lengths of the female were 5.27±0.79 μm and 2.24-7.70 μm respectively (table 3b).

The ANOVA test showed that the population of sarcomere lengths of the whole muscle of the male was significantly different from that of the female ($p < 0.001$). The ANOVA also showed that the population of the dorsal region was significantly different from that of the ventral region within the muscles of both the male and the female, but with different levels of probability ($p < 0.001$ for the male and $p = 0.003$ for the female). This and the difference between F ratio for sarcomere lengths within the muscle of the male (DF=11 and F=112.48) and that of the female (DF=11 and F=33.30) may suggest that the muscle of the female is less heterogeneous. The range and STD of the muscles also showed that variation of sarcomere lengths within the male was greater than that of the female. The wide range and STD of sarcomere lengths of fibres in each individual part showed that, the variation of sarcomere lengths within each individual part was great (table 3a, b).

The significant differences between parts and the range and STD of sarcomere lengths within the whole muscles and within each individual part suggest that the muscles were a mixture of fibres with significantly shorter and longer sarcomeres.

The Z lines of the all muscle fibres were wavy, however the Z lines of some fibres were more regular and clearer than others (fig. 20, 21).

3.3.2. Cross sectional area of the muscle fibres

The fibres with the smallest and largest cross sectional area

Fig. 20: Longitudinal section of the muscle fibre which has relatively regular, straight and thin Z lines that is typical of fast muscle fibres. X 12000.

Fig. 21: Longitudinal section of the muscle fibre which has relatively irregular, wavy and thick Z lines that is typical of slow muscle fibres. X 7500

within the muscle of the male were found in, VPP and DDP parts with mean cross sectional areas of 2,543±748 μm^2 and 15,231±4,348 μm^2 respectively (table 4a). The ANOVA test for the cross sectional areas showed that the population was not homogeneous and that some muscle parts within the male were significantly different from others ($p < 0.001$). The T-test between individual parts showed that more than 80% of the muscle parts from the male were significantly different from each other ($p=0.05$).

The ANOVA test showed that the population of the dorsal region of the male, with mean fibres cross sectional area of 10603±4348 μm^2, was significantly different from that of the ventral region, with mean of 4167±1712 μm^2, indicating that some of the parts of either region are different from all of the parts of the other region. ($p < 0.001$). The muscle parts ranked with regard to the mean cross sectional area of their fibres (table, 4a; fig. 22) showed that, the fibres of the dorsal parts with the exception of DDA had larger mean cross sectional areas than the ventral parts. The range and STD of the fibres area within the dorsal region were greater than those of ventral region. The distal parts are with medium and larger fibres and the proximal parts with smaller, medium and larger fibres. The mean and range of the cross sectional area of fibres within the whole muscle of the male were, 7,385±4,165 μm^2 and 582-25,550 μm^2 (table, 5a).

The fibres with the smallest and largest cross sectional area

Table 4: Mean cross section area of fibres in the 12 parts of the flexor tibialis muscle of a) male and b) female ranked from the largest area to the smallest. Significant difference between pairs was tested at p=0.05. (x) indicates no significant difference, (-) significant difference.

a)

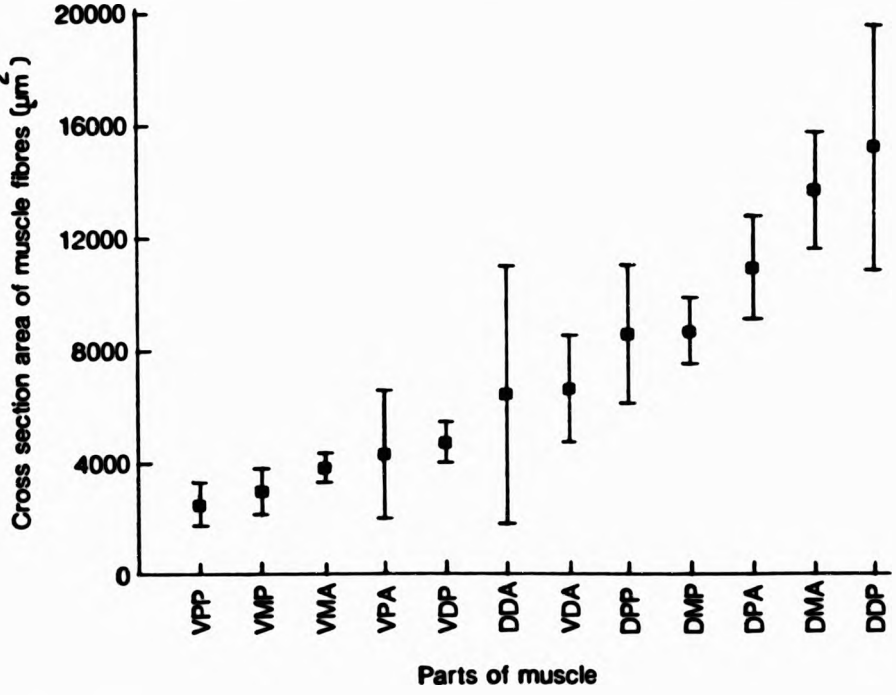
	DDP	DMA	DPA	DMP	DPP	VDA	DDA	VDP	VPA	VMA	VMP	VPP	MEAN±STD (μm^2)
DDP		x	-	-	-	-	-	-	-	-	-	-	15,231±4,348
DMA	x		-	-	-	-	-	-	-	-	-	-	13,681±2,091
DPA	-	-		-	-	-	-	-	-	-	-	-	10,979±1,817
DMP	-	-	-		x	-	x	-	-	-	-	-	8,696±1,194
DPP	-	-	-	x		-	x	-	-	-	-	-	8,597±2,470
VDA	-	-	-	-	-		x	-	-	-	-	-	6,625±1,890
DDA	-	-	-	x	x	x		x	x	-	-	-	6,432±4,581
VDP	-	-	-	-	-	-	x		x	-	-	-	4,725± 723
VPA	-	-	-	-	-	-	x	x		x	x	-	4,276±2,270
VMA	-	-	-	-	-	-	-	-	x		-	-	3,841± 477
VMP	-	-	-	-	-	-	-	-	x	-		-	2,990± 827
VPP	-	-	-	-	-	-	-	-	-	-	-		2,543± 748

b)

	VDP	DPA	DDA	VMP	VPA	VDA	DPP	VPP	DMP	DDP	DMA	VMA	MEAN+/-STD (μm^2)
VDP		-	-	-	-	-	-	-	-	-	-	-	9,648±1,851
DPA	-		x	x	x	x	-	-	-	-	-	-	7,885±2,285
DDA	-	x		x	x	x	x	-	-	-	-	-	7,269±1,127
VMP	-	x	x		x	x	x	-	-	-	-	-	7,076±1,350
VPA	-	x	x	x		x	x	-	-	-	-	-	6,812±1,193
VDA	-	x	x	x	x		x	-	-	-	-	-	6,787±1,412
DPP	-	-	x	x	x	x		-	-	-	-	-	6,713± 528
VPP	-	-	-	-	-	-	-		x	-	-	-	5,896± 772
DMP	-	-	-	-	-	-	-	x		x	x	-	5,504± 84
DDP	-	-	-	-	-	-	-	-	x		x	-	5,414± 583
DMA	-	-	-	-	-	-	-	-	x	x		-	5,330± 766
VMA	-	-	-	-	-	-	-	-	-	-	-		4,644± 555

Fig. 22: Showing the muscle parts from the male ranked, according to their mean cross sectional area of fibres.

Fig. 23: Showing the muscle parts from the female ranked, according to their mean cross sectional area of fibres.


Cross section area of muscle fibres (μm²)
20000
16000
12000
8000
4000
0
VPP
VMP
VMA
VPA
VDP
DDA
VDA
DPP
DMP
DPA
DMA
DDP
Parts of muscle

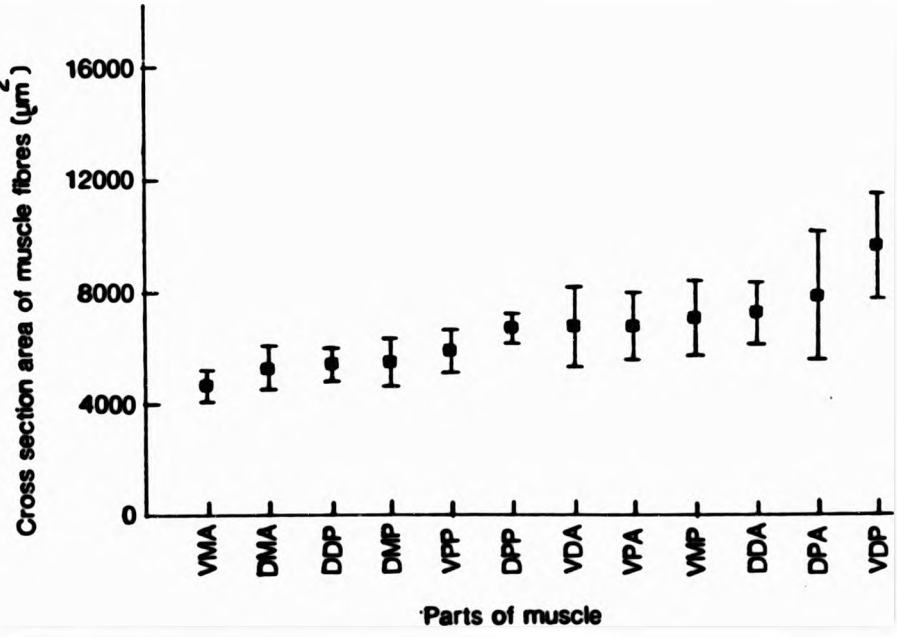

Cross section area of muscle fibres (μm²)
16000
12000
8000
4000
0
VMA
DMA
DDP
DMP
VPP
DPP
VDA
VPA
VMP
DDA
DPA
VDP
Parts of muscle

Table 5: Mean, standard deviation and range of cross sectional area of fibres within the 12 parts and the flexor tibialis muscle of a) male and b) female. The numbers in the final column show the rank position for cross sectional area of the fibres (mean) of the 12 muscle parts. Number (1) indicates the largest and number (12) the smallest cross section area.

a)

MUSCLE PARTS	MEAN±STD (μm^2)	RANGE (μm^2)	RANK
DDA	6,432±4,581	582-13,382	7
DDP	15,231±4,348	7,525-25,550	1
DMA	13,681±2,091	9,218-17,119	2
DMP	8,696±1,194	6,480-10,591	4
DPA	10,979±1,817	7,960-15,522	3
DPP	8,597±2,470	4,985-14,906	5
VDA	6,625±1,890	3,284-11,135	6
VDP	4,725± 723	2,765- 6,296	8
VMA	3,841± 477	2,834- 4,739	10
VMP	2,990± 827	1,620- 5,418	11
VPA	4,276±2,270	2,874-11,940	9
VPP	2,543± 748	1,028- 4,580	12
WHOLE MUSCLE	7,385±4,165	582-25,550	

b)

MUSCLE PARTS	MEAN±STD (μm^2)	RANGE (μm^2)	RANK
DDA	7,269±1,127	5,975-10,425	3
DDP	5,414± 583	4,651- 7,153	10
DMA	5,330± 766	4,121- 7,195	11
DMP	5,504± 842	2,407- 6,613	9
DPA	7,885±2,285	5,235-15,027	2
DPP	6,713± 528	5,949- 7,781	7
VDA	6,787±1,412	4,593-10,042	6
VDP	9,648±1.851	6,488-12,201	1
VMA	4,644± 555	3,787- 5,691	12
VMP	7,076±1,350	4,830- 9,478	4
VPA	6,812±1,193	4,645- 9,081	5
VPP	5,896± 772	4,901- 7,409	8
WHOLE MUSCLE	6,581±1,357	2,407-15,027	

within the muscle of the female were found in VMA and VDP parts, with mean cross sectional areas of 4,644±555 μm^2 and 9,648±1,851 μm^2 respectively (table 4b). The ANOVA test for cross sectional areas of the muscle parts from the female showed that, the population was not homogeneous and that some muscle parts were significantly different from others ($p < 0.001$). The T-test between individual parts showed that, most parts of the muscle from the female were significantly different from each other ($p=0.05$).

The ANOVA test showed that the population of the dorsal region of the female, with mean fibres cross sectional area of 6352±1579 μm^2, was significantly different from that of the ventral region, with mean of 6810±1768 μm^2, indicating that some of the parts of either region are different from all of the parts of the other region ($p=0.007$). Most dorsal parts have smaller fibres (mean cross sectional area) than ventral ones (table 4b). The muscle parts ranked with regard to the mean cross sectional area of their fibres (table 4b; fig. 23) showed that, the parts with smaller cross sectional area were significantly different to the parts with medium and larger cross sectional areas ($p=0.05$). The fibres with larger and smaller cross sectional area did not tend to be in a particular region (the proximal, medial and distal regions were contained fibres with larger and smaller cross sectional areas) of the muscle. The mean and range of the cross sectional area of fibres within the muscle of the female were, 6,581±1,357 μm^2 and 2,407-15,027 μm^2 respectively (table 5b).

The ANOVA test showed that the population of the cross sectional areas of fibres from the whole muscle of the male was significantly different from that of the female ($p < 0.001$). The ANOVA showed that the population in the dorsal region was significantly different from that in the ventral region within the muscles of both the male and the female, but with different level of probability and F ratios ($p < 0.001$ and $F = 479.14$ for the male; $p = 0.007$ and $F = 7.41$ for the female). The variation of the muscle from the male was considerably greater than that of the female. These may suggest that the muscle of the female is less heterogeneous than that of the male. The great range and STD of cross sectional area of fibres within each individual part and within the whole muscle of the male and female (table 5a b) showed that, variation between the cross sectional area of fibres within the muscles was great.

The significant differences between the cross sectional area of fibres within the muscle parts of both the male and female, the wide range and STD of the cross sectional area of the fibres within each individual part and within the whole muscles showed that, the muscle of the male and female were a mixture of fibres with significantly smaller and larger cross sectional areas.

The muscle of the male and the female contained fibres with different shapes (strap like, polygonal and irregular shapes) but most fibres were polygonal shaped.

3.3.3. The ratio of thin to thick filaments

The parts with the lowest and highest ratio of thin to thick filaments within the muscle of the male were, VMP with the mean ratio of 3.51±0.69:1 and VPP with the mean ratio of 5.65±1.12:1 (table 6a). The ANOVA test for the ratios showed that the population was not homogeneous and that some muscle parts within the male were significantly different from others ($p < 0.001$). The T-test between individual parts showed that most parts of the male muscle were significantly different from each other ($p=0.05$). The muscle parts ranked with regard to the mean ratio of their fibres (table 6a; fig. 24) showed that, the parts with lower ratio were significantly different from parts with medium and higher mean ratios.

The ANOVA test showed that the population of the dorsal region of the male, with mean ratios of 5.23±0.86:1, was significantly different from that of the ventral region, with mean of 4.48±1.04:1, this indicates that some of the parts of either region are different from all of the parts from the other region ($p < 0.001$). The ventral parts with the exception of VPP, which had the highest ratio, had lower ratios. The distal and medial parts tended to have lower, medium and higher thin to thick filament ratios and proximal parts medium to higher ratios. The mean and range of filament ratio of the whole muscle from the male were, 4.85±1.03:1 and 2.77-8.68:1 (table 7a).

The parts with lowest and highest thin to thick filaments

Table 6: Mean thin to thick filament ratio of fibres in the 12 parts of the flexor tibialis muscle of a) male and b) female ranked from the lowest ratio to the highest. Significant difference between pairs was tested at $p = 0.05$. (x) indicates no significant difference, (-) significant difference.

a)

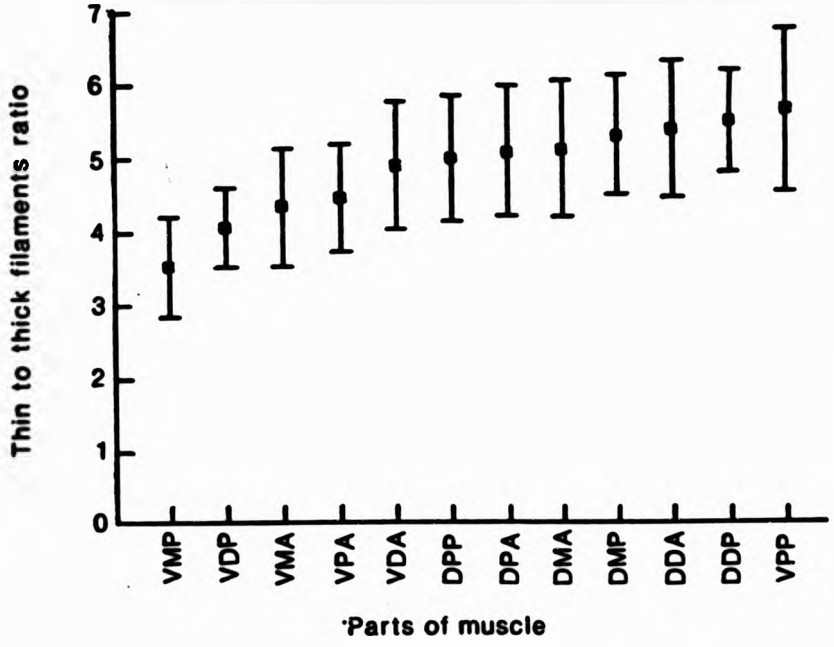
	VMP	VDP	VMA	VPA	VDA	DPP	DPA	DMA	DMP	DDA	DDP	VPP	MEAN±STD
VMP		-	-	-	-	-	-	-	-	-	-	-	3.51±0.69:1
VDP	-		x	x	-	-	-	-	-	-	-	-	4.05±0.54:1
VMA	-	x		x	-	-	-	-	-	-	-	-	4.32±0.81:1
VPA	-	x	x		x	-	-	-	-	-	-	-	4.45±0.73:1
VDA	-	-	-	x		x	x	x	x	x	-	-	4.89±0.87:1
DPP	-	-	-	-	x		x	x	x	x	-	-	4.99±0.87:1
DPA	-	-	-	-	x	x		x	x	x	x	x	5.08±0.89:1
DMA	-	-	-	-	x	x	x		x	x	x	x	5.11±0.93:1
DMP	-	-	-	-	x	x	x	x		x	x	x	5.31±0.83:1
DDA	-	-	-	-	x	x	x	x	x		x	x	5.39±0.94:1
DDP	-	-	-	-	-	-	x	x	x	x		x	5.50±0.70:1
VPP	-	-	-	-	-	-	x	x	x	x	x		5.65±1.12:1

b)

	VPP	VDA	DDP	DMP	VMA	DPA	VPA	VMP	DMA	DPP	VDP	DDA	MEAN±STD
VPP		x	-	-	-	-	-	-	-	-	-	-	4.02±0.61:1
VDA	x		-	-	-	-	-	-	-	-	-	-	4.17±0.48:1
DDP	-	-		x	x	x	-	-	-	-	-	-	4.54±0.35:1
DMP	-	-	x		x	x	x	-	-	-	-	-	4.70±0.56:1
VMA	-	-	x	x		x	x	-	-	-	-	-	4.72±0.48:1
DPA	-	-	x	x	x		x	x	-	-	-	-	4.74±0.66:1
VPA	-	-	-	x	x	x		x	x	x	-	-	4.98±0.78:1
VMP	-	-	-	-	-	x	x		x	x	-	-	5.02±0.18:1
DMA	-	-	-	-	-	-	x	x		x	x	-	5.21±0.75:1
DPP	-	-	-	-	-	-	x	x	x		x	x	5.46±1.16:1
VDP	-	-	-	-	-	-	-	-	x	x		x	5.70±0.91:1
DDA	-	-	-	-	-	-	-	-	-	x	x		5.72±0.60:1

Fig. 24: Showing the muscle parts from the male ranked, according to their mean thin to thick filament ratios of fibres.

Fig. 25: showing the muscle parts from the female ranked, according to their mean thin to thick filament ratios of fibres.


Thin to thick filaments ratio
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1
2
3
4
5
6
7
VMP
VDP
VMA
VPA
VDA
DPP
DPA
DMA
DMP
DDA
DDP
VPP
Parts of muscle

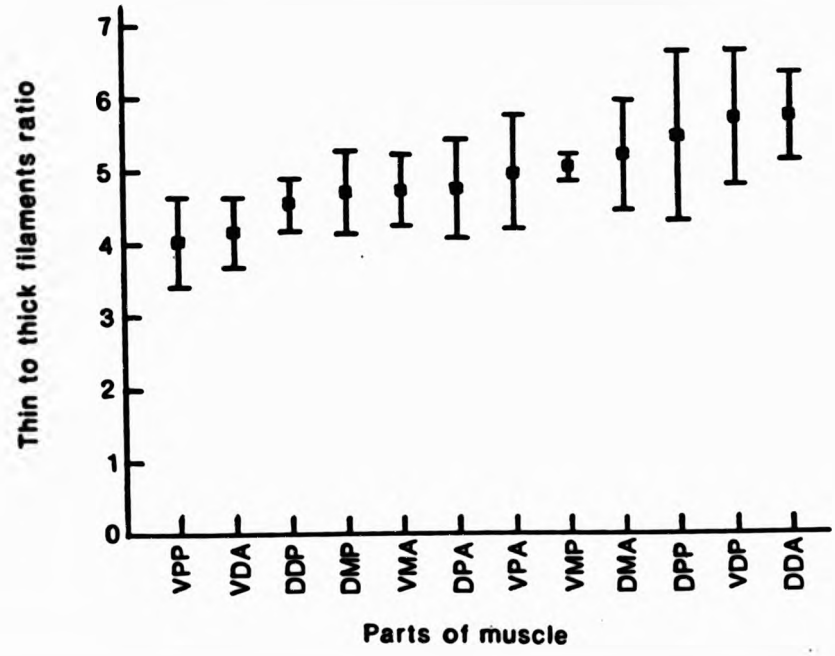

Thin to thick filaments ratio
0
1
2
3
4
5
6
7
VPP
VDA
DDP
DMP
VMA
DPA
VPA
VMP
DMA
DPP
VDP
DDA
Parts of muscle

Table 7: Mean, standard deviation and range of ratio of thin to thick filaments of fibres within the 12 parts and the felxor tibialis muscle from a) male and b) female. The numbers in the final column shows the rank position for ratio (mean) of the 12 muscle parts. Number (1) indicates the lowest and number (12) the highest ratio.

a)

MUSCLE PARTS	MEAN±STD	RANGE	RANK
DDA	5.39±0.94:1	3.95-8.10	10
DDP	5.50±0.70:1	4.18-6.48	11
DMA	5.11±0.93:1	3.32-7.33	8
DMP	5.31±0.83:1	3.65-6.81	9
DPA	5.08±0.89:1	3.16-6.47	7
DPP	4.99±0.87:1	3.57-6.46	6
VDA	4.89±0.87:1	3.13-6.09	5
VDP	4.05±0.54:1	3.03-5.69	2
VMA	4.32±0.81:1	2.87-5.80	3
VMP	3.51±0.69:1	2.77-5.17	1
VPA	4.44±0.73:1	3.27-5.86	4
VPP	5.65±1.12:1	4.21-8.68	12
Whole muscle	4.85±1.03:1	2.77-8.68	

b)

MUSCLE PARTS	MEAN±STD	RANGE	RANK
DDA	5.72±0.60:1	4.37-6.88	12
DDP	4.54±0.35:1	4.00-5.19	3
DMA	5.21±0.75:1	3.59-5.91	9
DMP	4.70±0.56:1	3.89-5.93	4
DPA	4.74±0.66:1	3.36-5.69	6
DPP	5.46±1.16:1	3.50-7.86	10
VDA	4.17±0.48:1	2.86-4.83	2
VDP	5.70±0.91:1	4.09-7.25	11
VMA	4.72±0.48:1	4.10-5.75	5
VMP	5.02±0.18:1	4.68-5.38	8
VPA	4.98±0.78:1	3.21-6.25	7
VPP	4.02±0.61:1	3.06-5.14	1
WHOLE MUSCLE	4.92±0.84:1	2.86-7.86	

ratio within the muscle of the female were, VPP with the mean ratio of 4.02±0.61:1 and DDA with the mean ratio of 5.72±0.60:1 (table 6b). The ANOVA test for the ratios showed that the population was not homogeneous and that some muscle parts within the female were significantly different from others ($p < 0.001$). The T-test between individual parts showed that there were significant differences ($p=0.05$) between the majority of the parts from the muscle of the female. The muscle parts ranked with respect to the mean ratios of their fibres (table 6b; fig. 25) showed that, the only the parts which were juxtaposed within the ranking did not tend to be significantly different from each other. The parts with lower or higher ratio did not tend to be in a particular region (the proximal, medial and distal regions were contained fibres with higher and lower ratios) of the muscle.

The ANOVA test showed that the population of the dorsal region of the female, with mean ratios of 5.06±0.83:1, was significantly different from that of the ventral region, with mean of 4.77±0.82:1, this indicates that some of the parts of either region are different from all of the parts of the other region ($p=0.006$). The dorsal parts had usually higher ratio than the ventral parts. The mean and range of thin to thick filaments ratio within the female were, 4.92±0.84:1 and 2.86-7.86:1 respectively (table 7b).

The ANOVA test for the ratios showed that population of the whole muscle of the male and the female were not significantly

different from each other (p=0.465). The ANOVA also showed that the population of the dorsal region was significantly different from that of the ventral region within the muscles of both the male and the female, but with different levels of probability (p <0.001 for the male and p=0.006 for the female). The STD and range of the ratio for the muscle from the male and the female showed that the variation within the male was greater than that of the female (table 7a, b). These may suggest that the fibres population and distribution in the muscle of the female is less heterogeneous. The significant differences between parts, the wide range of ratio within the whole muscles and also within each individual parts showed that the muscles from both the male and female were a mixture of fibres with significantly lower and higher thin to thick filaments ratio.

3.3.4. Thick filaments number

The parts with the lowest and highest number of thick filaments within the muscle of the male were, DDA and VMP with mean number per μm^2 of 471.2±74.9 and 848.8±102.4 respectively (table 8a). The ANOVA test for the thick filament number showed that the population was not homogeneous and that some muscle parts within the male were significantly different from others (p <0.001). The T-test between individual parts showed that most parts of the muscle from the male were significantly different from each other (p=0.05). The muscle parts ranked with respect to the mean number of thick filaments of their fibres (table 8a; fig. 26) showed that, the parts which were

Table 8: Mean thick filament number per μm^2 of fibres in the 12 parts of the flexor tibialis muscle of a) male and b) female ranked from the highest number to the lowest. Significant difference between pairs was tested at p=0.05. (x) indicates no significant difference, (-) significant difference.

a)

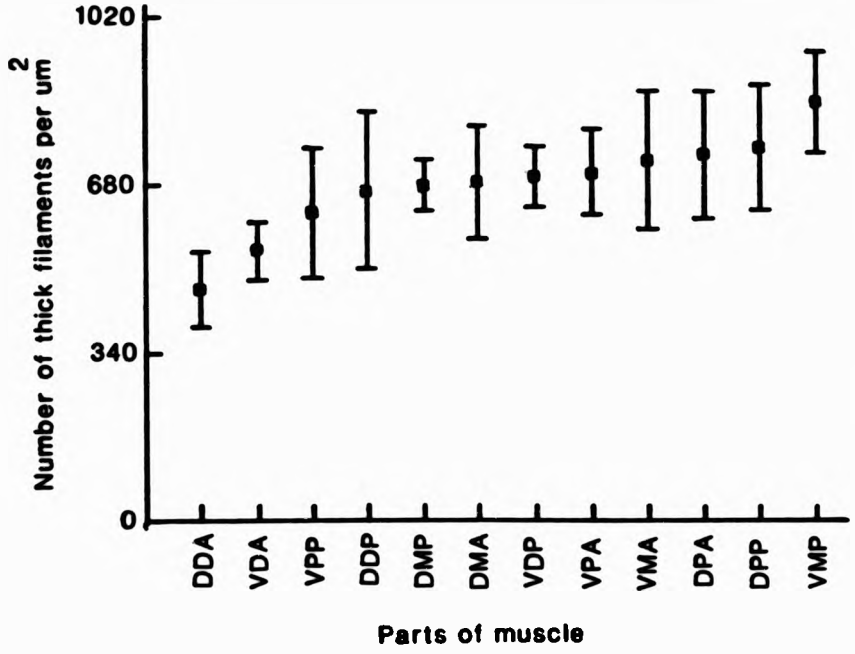
	VMP	DPP	DPA	VMA	VPA	VDP	DMA	DMP	DDP	VPP	VDA	DDA	MEAN±STD
VMP		-	-	-	-	-	-	-	-	-	-	-	848.8±102.4
DPP	-		x	x	x	x	x	-	x	-	-	-	756.3±125.4
DPA	-	x		x	x	x	x	x	x	-	-	-	741.3±128.3
VMA	-	x	x		x	x	x	x	x	-	-	-	730.0±138.0
VPA	-	x	x	x		x	x	x	x	-	-	-	706.3± 88.1
VDP	-	x	x	x	x		x	x	x	-	-	-	697.5± 61.2
DMA	-	x	x	x	x	x		x	x	x	-	-	683.8±113.3
DMP	-	-	x	x	x	x	x		x	x	-	-	678.8± 51.5
DDP	-	x	x	x	x	x	x	x		x	x	-	670.0±158.9
VPP	-	-	-	-	-	-	x	x	x		-	-	623.8±130.4
VDA	-	-	-	-	-	-	-	-	x	-		-	548.8± 57.6
DDA	-	-	-	-	-	-	-	-	-	-	-		471.2± 74.9

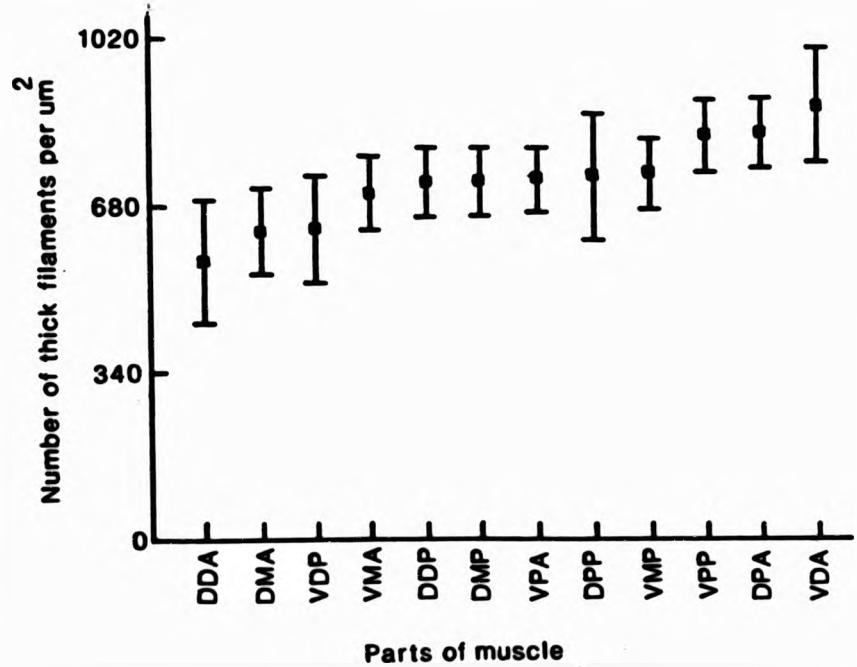
b)

	VDA	DPA	VPP	VMP	DPP	VPA	DMP	DDP	VMA	VDP	DMA	DDA	MEAN+/-STD
VDA		x	-	-	-	-	-	-	-	-	-	-	881.3±114.4
DPA	x		x	-	-	-	-	-	-	-	-	-	823.8± 71.9
VPP	-	x		-	-	-	-	-	-	-	-	-	817.5± 73.5
VMP	-	-	-		x	x	x	x	x	-	-	-	741.3± 70.4
DPP	-	-	-	x		x	x	x	x	-	-	-	736.3±127.6
VPA	-	-	-	x	x		x	x	x	-	-	-	728.8± 65.5
DMP	-	-	-	x	x	x		x	x	-	-	-	726.3± 67.1
DDP	-	-	-	x	x	x	x		x	-	-	-	723.8± 70.0
VMA	-	-	-	x	x	x	x	x		-	-	-	702.5± 74.7
VDP	-	-	-	-	-	-	-	-	-		x	x	631.3±109.1
DMA	-	-	-	-	-	-	-	-	-	x		x	627.5± 87.7
DDA	-	-	-	-	-	-	-	-	-	x	x		565.0±124.4

Fig. 26: Showing the muscle parts from the male ranked, according to their mean thick filaments number per μm^2 of fibres.

Fig. 27: Showing the muscle parts from the female ranked, according to their mean thick filaments number per μm^2 of fibres.


1020
680
340
0
Number of thick filaments per um 2
DDA
VDA
VPP
DDP
DMP
DMA
VDP
VPA
VMA
DPA
DPP
VMP
Parts of muscle


1020
680
340
0
Number of thick filaments per um 2
DDA
DMA
VDP
VMA
DDP
DMP
VPA
DPP
VMP
VPP
DPA
VDA
Parts of muscle

not significantly different tended to have a medium to high number of thick filaments.

The ANOVA test showed that population of the dorsal region of the male, with the mean thick filament number per μm^2 of 666.9±146, was not significantly different from that of the ventral region, with mean of 692.5±135.8, this indicates that none of the parts of either region is different from all of the parts of the other region ($p=0.105$). Whereas the T-test showed that some of the dorsal parts were significantly different from some of the ventral parts ($p=0.05$). Most ventral parts of the muscle from the male had a higher thick filament numbers than the dorsal ones. The proximal parts tended to have higher and medium thick filaments number. the medial parts had the medium to higher numbers and the distal parts had the lower to medium thick filament numbers. The mean and range of thick filament number per μm^2 within the muscle from the male were, 679.7±141.3 and 250-1,025 (table 9a).

The parts with the lowest and highest number of thick filaments within the muscle of the female were, DDA and VDA with mean number per μm^2 of 565.0±124.4 and 881.3±114.4 respectively (table 8b). The ANOVA test for the thick filament number showed that the population was not homogeneous and that some muscle parts within the female were significantly different from others ($p < 0.001$). The T-test between individual parts showed that there were significant differences between the majority of the muscle parts of the

Table 9: Mean, standard deviation and range of thick filaments number per μm^2 of fibres within the 12 parts and the flexor tibialis muscle of a) male and b) female. The numbers in the final column shows the rank position for thick filaments number (mean) of the 12 muscle parts. Number (1) indicates the largest and number (12) the smallest mean thick filaments number.

a)

MUSCLE PARTS	MEAN±STD	RANGE	RANK
DDA	471.3± 74.9	250- 625	12
DDP	670.0±158.9	425- 975	9
DMA	683.8±113.3	525- 925	7
DMP	678.8± 51.5	575- 775	8
DPA	741.3±128.3	475- 950	3
DPP	756.2±125.4	550-1,000	2
VDA	548.8± 57.6	425- 625	11
VDP	697.5±61.2	575- 825	6
VMA	730.0±138.0	500- 975	4
VMP	848.8±102.4	650-1,025	1
VPA	706.3± 88.1	550- 900	5
VPP	623.8±130.4	475- 975	10
WHOLE MUSCLE	679.7±141.3	250-1,025	

b)

MUSCLE PARTS	MEAN±STD	RANGE	RANK
DDA	565.0±124.4	400- 800	12
DDP	723.8± 70.0	575- 875	8
DMA	627.5± 87.7	525- 850	11
DMP	726.3± 67.1	575- 850	7
DPA	823.8± 71.9	675- 975	2
DPP	736.3±127.6	550-1,025	5
VDA	881.3±114.4	600-1,050	1
VDP	631.3±109.1	450- 875	10
VMA	702.5± 74.7	550- 800	9
VMP	741.3± 70.4	600- 850	4
VPA	728.8± 65.5	575- 850	6
VPP	817.5± 73.5	675- 925	3
WHOLE MUSCLE	725.4±123.3	400-1,050	

female (p=0.05). The muscle parts ranked with regard to the mean number of thick filaments of their fibres (table 8b; fig. 27) showed that, the parts which were not significantly different tended to have medium number of thick filaments.

The ANOVA test showed that the population of the dorsal region of the female, with mean thick filament number per μm^2 of 700.4±124.8, was significantly different from that of the ventral region, with mean of 750.4±117.1, this indicates that some of the parts of either region are significantly different from all of the other region (p=0.001). Most ventral muscle parts of the female had higher numbers of thick filaments than dorsal ones. The distal parts tended to have higher and lower thick filaments number, the medial parts lower to medium and the proximal parts tended to have higher and medium thick filaments number. The mean and range of thick filaments number per μm^2 of the female were, 725.4±123.3 and 400-1,050 respectively (table 9b).

The ANOVA test for the thick filaments number showed that the population of the whole muscle of the male was significantly different from that of the female ($p < 0.001$). The ANOVA also showed that the population of the dorsal region was significantly different from that in the ventral region within the muscle of the female (p=0.001), however the population of the dorsal region of the male was not significantly different from that of the ventral region (p=0.105). This suggests that the muscle of the male is less heterogeneous.

The range and STD of thick filaments number within the whole muscle of the male and the female showed that, the variation between thick filaments number of fibres within the muscle of the male is greater than that of the female (table 9a, b). The significant differences between the mean thick filaments number within the parts, the great range within each individual part and within the whole muscle of the male and female showed that, the muscles were a mixture of fibres with significantly higher and lower thick filaments number.

3.3.5. Thin filaments number

The muscle parts with the lowest and highest number of thin filaments from the male were; DDA and DPA with mean number of thin filaments per μm^2 of 2,514±482 and 3,688±512 respectively (table 10a). The ANOVA test for the thin filaments number showed that the population was not homogeneous and that some muscle parts within the male were significantly different from others ($p < 0.001$). The T-test between individual parts showed that most parts of the muscle of the male were significantly different from each other ($p=0.05$). The muscle parts ranked with respect to the mean thin filaments number of their fibres (table 10a; fig. 28) showed that, the muscle parts with lower number of thin filaments tended to be significantly different from the parts with higher number.

The ANOVA test showed that the population of the dorsal region of the male, with the mean thin filament number per μm^2 of 3423±670, was significantly different from that of the ventral

Table 10: Mean thin filament number per μm^2 of fibres in the 12 parts of the flexor tibialis muscle of a) male and b) female ranked from the lowest to the highest. Significant difference between pairs was tested at p=0.05. (x) indicates no significant difference, (-) significant difference.

a)

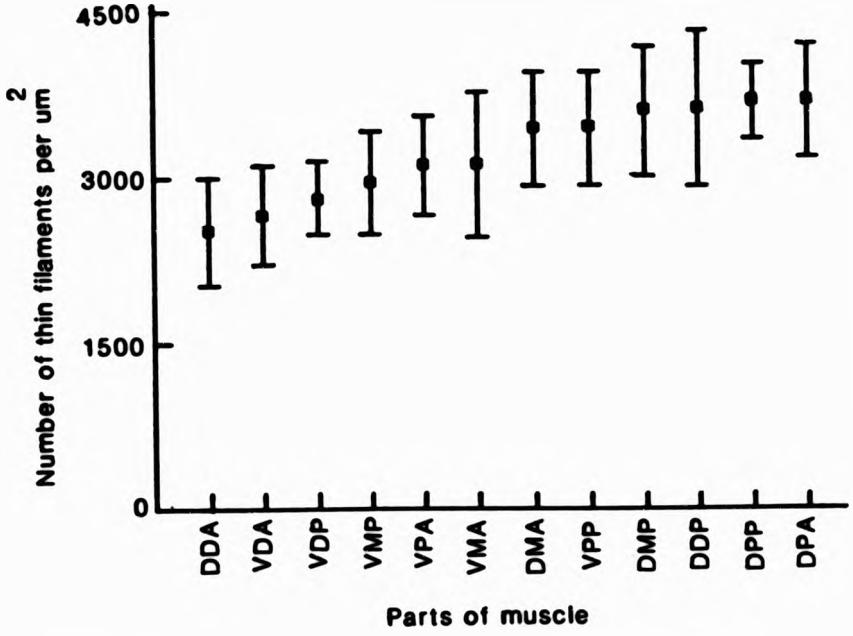
	DDA	VDA	VDP	VMP	VPA	VMA	DMA	VPP	DMP	DDP	DPP	DPA	MEAN±STD
DDA		x	-	-	-	-	-	-	-	-	-	-	2,514±482
VDA	x		x	x	-	-	-	-	-	-	-	-	2,659±448
VDP	-	x		x	-	x	-	-	-	-	-	-	2,813±328
VMP	-	x	x		x	x	-	-	-	-	-	-	2,949±465
VPA	-	-	-	x		x	-	-	-	-	-	-	3,106±447
VMA	-	-	x	x	x		x	x	-	-	-	-	3,113±660
DMA	-	-	-	-	-	x		x	x	x	x	x	3,435±514
VPP	-	-	-	-	-	x	x		x	x	x	x	3,441±513
DMP	-	-	-	-	-	-	x	x		x	x	x	3,599±585
DDP	-	-	-	-	-	-	x	x	x		x	x	3,619±707
DPP	-	-	-	-	-	-	x	x	x	x		x	3,684±343
DPA	-	-	-	-	-	-	x	x	x	x	x		3,688±512

b)

	DDA	DMA	VPP	DDP	VMA	DMP	VDP	VPA	VDA	VMP	DPA	DPP	MEAN±STD
DDA		x	x	x	x	x	-	-	-	-	-	-	3,171±393
DMA	x		x	x	x	x	-	-	-	-	-	-	3,213±249
VPP	x	x		x	x	x	-	-	-	-	-	-	3,270±442
DDP	x	x	x		x	x	-	-	-	-	-	-	3,285±386
VMA	x	x	x	x		x	-	-	-	-	-	-	3,305±363
DMP	x	x	x	x	x		x	x	x	-	-	-	3,409±440
VDP	-	-	-	-	-	x		x	x	-	-	-	3,518±295
VPA	-	-	-	-	-	x	x		x	x	x	x	3,616±567
VDA	-	-	-	-	-	x	x	x		x	x	x	3,650±496
VMP	-	-	-	-	-	-	-	x	x		x	x	3,719±331
DPA	-	-	-	-	-	-	-	x	x	x		x	3,914±696
DPP	-	-	-	-	-	-	-	x	x	x	x		3,920±565

Fig. 28: Showing the muscle parts from the male ranked, according to their mean thin filament number per μm^2 of fibres.

Fig. 29: Showing the muscle parts from the female ranked, according to their mean thin filament number per μm^2 of fibres.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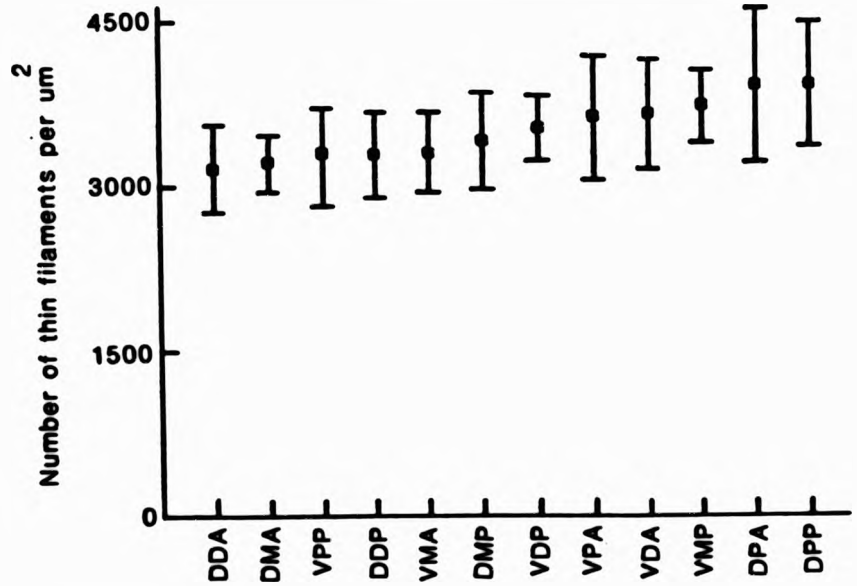
3000
1500
0
Number of thin filaments per um 2
DDA
VDA
VDP
VMP
VPA
VMA
DMA
VPP
DMP
DDP
DPP
DPA
Parts of muscle


4500
3000
1500
0
Number of thin filaments per um 2
DDA
DMA
VPP
DDP
VMA
DMP
VDP
VPA
VDA
VMP
DPA
DPP

region, with the mean number of 3013±538, this indicates that some of the parts of either region are different from all of the parts of the other region ($p < 0.001$). The most ventral parts had lower numbers of thin filaments. The distal parts tended to have lower thin filaments number, the medial parts to have medium number and the proximal parts medium and higher thin filaments number. The mean and range of thin filaments number per μm^2 of the whole muscle from the male were 3,218±640.0 and 1,775-5,200 respectively (table 11a).

The parts with lowest and highest number of thin filaments within the muscle from the female were, DDA and DPP with the mean thin filaments number per μm^2 of 3,171±393 and 3,920±565 respectively (table 10b). The ANOVA test for the thin filaments number showed that the population was not homogeneous and that some muscle parts within the female were significantly different from others ($p < 0.001$). The T-test between individual parts showed that the majority of the muscle parts of the female were significantly different from each other ($p=0.05$). The muscle parts ranked with regard to the mean thin filaments number of their fibres (table 10b; fig. 29) showed that, the parts with lower numbers of thin filaments were significantly different from the parts with higher numbers.

The ANOVA test showed that the population of the dorsal region of the female, with the mean thin filament number per μm^2 of 3485±563, was not significantly different from that of the

Table 11: Mean, standard deviation and range of thin filaments number per μm^2 of fibres within the 12 parts and the flexor tibialis muscle of a) male and b) female. The numbers in the final column shows the rank position for thin filaments number (mean) of the 12 muscle parts. Number (1) indicates the lowest and number (12) the highest mean thin filaments number.

a)

MUSCLE PARTS	MEAN±STD	RANGE	RANK
DDA	2,514±482	1,775-3,425	1
DDP	3,619±707	2,550-5,200	10
DMA	3,435±514	2,325-4,500	7
DMP	3,599±585	2,375-4,600	9
DPA	3,688±512	2,525-4,700	12
DPP	3,684±343	3,125-4,325	11
VDA	2,659±448	1,875-3,500	2
VDP	2,813±328	2,100-3,700	3
VMA	3,113±660	2,150-4,200	6
VMP	2,949±465	2,075-3,750	4
VPA	3,106±447	2,450-3,825	5
VPP	3,441±513	2,575-4,525	8
WHOLE MUSCLE	3,218±640	1,775-5,200	

b)

MUSCLE PARTS	MEAN±STD	RANGE	RANK
DDA	3,171±393	2,450-3,900	1
DDP	3,285±386	2,725-4,375	4
DMA	3,213±249	2,750-3.675	2
DMP	3,409±440	2,400-4,275	6
DPA	3,914±696	2,475-5,375	11
DPP	3,920±565	2,900-5,000	12
VDA	3,650±496	2,900-4,625	9
VDP	3,518±295	3,025-4,000	7
VMA	3,305±363	2,375-4,050	5
VMP	3,719±331	3,125-4,175	10
VPA	3,616±567	2,250-4,600	8
VPP	3,270±442	2,525-4,025	3
WHOLE MUSCLE	3,499±509	2,250-5,375	

ventral region, with the mean number of 3513±451, this indicates that none of the parts of either region is different from all of the parts of the other region (p=0.644). Whereas the T-test showed that some dorsal parts were significantly different from some of the ventral parts (p=0.05). The two parts with the highest numbers of thin filaments, and the two parts with the lowest number were located in the dorsal regions. The distal parts tended to have lower and medium thin filaments number, the medial parts to have lower and medium and the proximal parts tended to have lower and higher thin filaments number. The mean and range of thin filaments number per μm^2 from the female were; 3,499±509.2 and 2,250-5,375 respectively (table 11b).

The ANOVA test showed that the population of the thin filaments number of the whole muscle of the male was significantly different from that of the female ($p < 0.001$). The ANOVA showed that the population of the dorsal region was significantly different from that of the ventral region within the muscle of the male ($p < 0.001$), but the population of the dorsal region was not significantly different from that of the ventral region in the female (p=0.644). This suggests that the muscle of the female is less heterogeneous.

The STD and range of thin filament number within the muscle of the male and female showed that, variation between thin filaments number of fibres within the muscle of the male was greater than that of the female (table, 11a, b). The

significant differences between the muscle parts, the range of thin filaments number of the whole muscle and within each individual part showed that, the muscle of the male and female were a mixture of fibres with significantly lower and higher number of thin filaments.

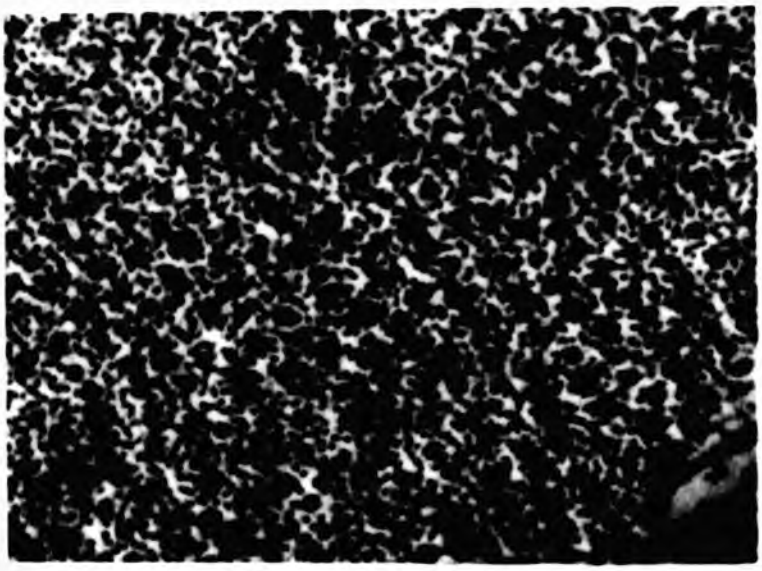
The arrangement of thick and thin filaments in some fibres was regular i.e. the thick filaments were arranged in a perfect square array and each one was surrounded by a circle of 9-12 thin filaments (fig. 30). In other fibres the arrangement and number of filaments were relatively irregular (fig. 31). The number of thin filaments around each thick filament were varied, in some areas thin filaments were relatively fewer than thick filaments (fig. 30), however in others thin filaments were abundant and less thick filaments present (fig. 32). Where each thick filament was surrounded by one quite clear circle of the thin filaments, there were 9-12 thin filaments (fig. 30).

3.3.6. Area, number and average size of mitochondria

The parts with the smallest and largest mitochondrial area within the muscle of the male were, VMP and DMA with the mean mitochondrial area of 5.79±2.18% and 11.73±1.97% (table 12a). The ANOVA test for the mitochondrial area showed that the population was not homogeneous and that some muscle parts within the male were significantly different from others ($p < 0.001$). The T-test between individual parts showed that most parts of the muscle of the male were significantly

Fig. 30: Cross section of a muscle fibre, showing the arrangement of thick and thin filaments. The thick filaments with high density are arranged regularly in a square and a clear circle of thin filaments surrounding each thick filament, typical of fast fibre. X 157,000.

Fig. 31: Cross section of a muscle fibre, showing the irregular arrangement of thick and thin filaments, which is typical of slow fibre. X 157,000.



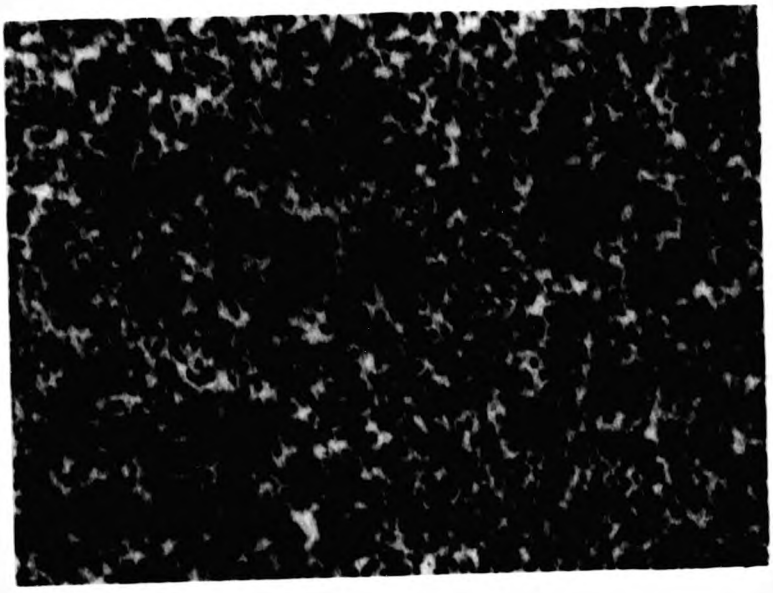


Fig. 32: Cross section of a muscle fibre, where less thick filaments exist. Which is typical of slow fibres. X 157,000

Table 12: Mean percentage area of mitochondria of fibres in the 12 parts of the flexor tibialis muscle from a) male b) female ranked from the lowest to the highest percentage. Significant difference between pairs was tested at p=0.05 (x) indicates no significant difference, (-) significant difference.

a)

	VMP	VDA	VMA	VDP	VPP	DMP	VPA	DDA	DPA	DPP	DDP	DMA	MEAN±STD (%)
VMP		x	x	x	-	-	x	-	-	-	-	-	5.79±2.18
VDA	x		x	x	-	x	x	-	-	-	-	-	5.85±2.58
VMA	x	x		x	x	x	x	-	-	-	-	-	6.16±2.30
VDP	x	x	x		x	x	x	x	-	-	-	-	6.83±2.93
VPP	-	-	x	x		x	x	x	-	-	-	-	7.27±1.38
DMP	-	x	x	x	x		x	x	-	-	-	-	7.42±2.56
VPA	x	x	x	x	x	x		x	x	-	-	-	7.82±4.90
DDA	-	-	-	x	x	x	x		x	x	-	-	9.00±4.01
DPA	-	-	-	-	-	-	x	x		x	x	x	10.00±3.36
DPP	-	-	-	-	-	-	-	x	x		x	x	10.52±2.51
DDP	-	-	-	-	-	-	-	-	x	x		x	11.48±2.21
DMA	-	-	-	-	-	-	-	-	x	x	x		11.73±1.97

b)

	DPP	DDP	DDA	DMA	DMP	VMA	VPP	VMP	DPA	VPA	VDP	VDA	MEAN±STD (%)
DPP		x	-	-	-	-	-	-	-	-	-	-	7.83±2.86
DDP	x		x	x	x	x	-	-	-	-	-	-	9.04±3.62
DDA	-	x		x	x	x	-	-	-	-	-	-	9.55±1.34
DMA	-	x	x		x	x	-	-	-	-	-	-	9.64±2.07
DMP	-	x	x	x		x	-	-	-	-	-	-	9.75±2.70
VMA	-	x	x	x	x		x	x	x	x	x	-	10.93±3.74
VPP	-	-	-	-	-	x		x	x	x	x	-	11.62±3.05
VMP	-	-	-	-	-	x	x		x	x	x	-	11.63±1.60
DPA	-	-	-	-	-	x	x	x		x	x	-	11.71±2.30
VPA	-	-	-	-	-	x	x	x	x		x	-	11.72±1.98
VDP	-	-	-	-	-	x	x	x	x	x		-	12.43±2.67
VDA	-	-	-	-	-	-	-	-	-	-	-		14.78±3.16

different from each other (p=0.05). The muscle parts ranked, from the lowest to the highest mean mitochondrial area of their fibres (table 12a; fig. 33) showed that, the parts with higher mitochondrial area tended to be significantly different from the parts with medium and lower mitochondrial area.

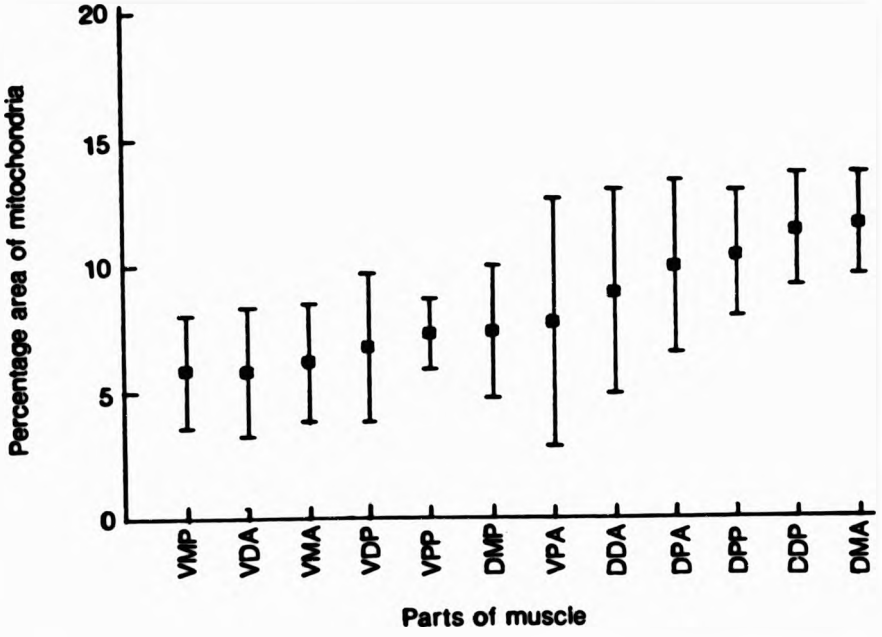
The ANOVA test showed that the population of the dorsal region of the male, with the mean mitochondrial area of 10.03±3.17%, was significantly different from that of the ventral region, with the mean area of 6.62±2.96%, this indicates that some of the parts of either region are different from all of the other parts of the other region ($p < 0.001$). The ventral parts of the male with exception of VPA had lower mitochondrial area than dorsal ones. The parts with highest and lowest mitochondrial area were located in the medial region of the muscle. The mean and range of the percentage area of mitochondria of the whole muscle from the male were, 8.32±3.50% and 0.79-19.15% respectively (table 13a).

The parts with the smallest and largest mitochondrial area within the muscle of the female were, DPP and VDA with the mean area of 7.83±2.86% and 14.78±3.16% respectively (table 12b). The ANOVA test for the mitochondrial area showed that the population was not homogeneous and that some muscle parts within the female were significantly different from others ($p < 0.001$). The T-test between each individual parts showed that the majority of the parts from the muscle of the female were significantly different from each other (p=0.05). The muscle

Fig. 33: Showing the muscle parts from the male ranked, according to their mean percentage area of mitochondria of fibres.

Fig. 34: Showing the muscle parts from the female ranked, according to their mean percentage area of mitochondria of fibres.

In the muscle of the male the dorsal parts have a higher percentage mitochondrial area which is opposite of the female where the ventral parts have higher percentage area.


Percentage area of mitochondria
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15
10
5
0
VMP
VDA
VMA
VDP
VPP
DMP
VPA
DDA
DPA
DPP
DDP
DMA
Parts of muscle

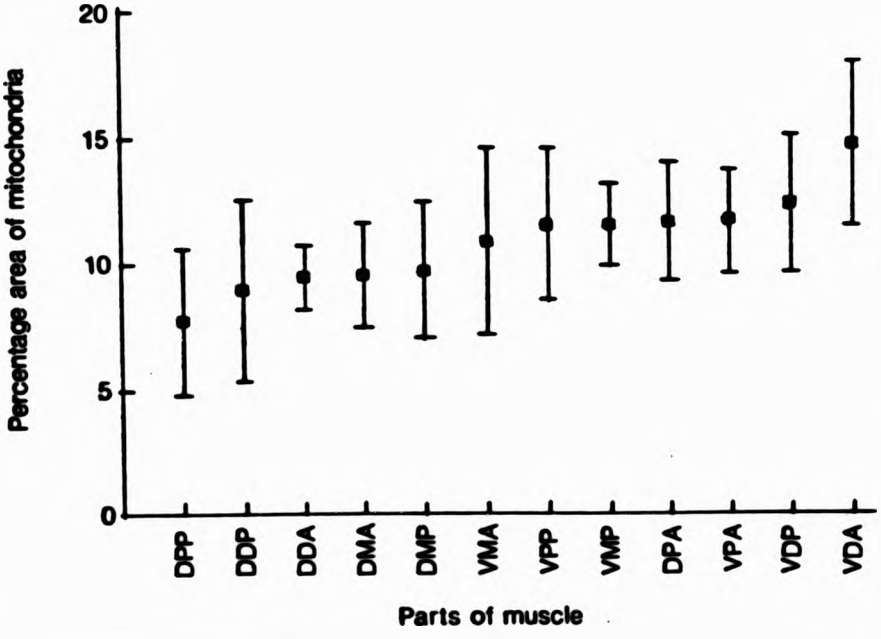

Percentage area of mitochondria
20
15
10
5
0
DPP
DDP
DDA
DMA
DMP
VMA
VPP
VMP
DPA
VPA
VDP
VDA
Parts of muscle

Table 13: Mean, standard deviation and range of percentage area of mitochondria within the 12 parts and the flexor tibialis muscle of a) male b) female. The numbers in the final column shows the rank position for mitochondrial area (mean) of the 12 muscle parts. Number (1) indicates the smallest and number (12) the largest mitochondrial area.

a)

MUSCLE PARTS	MEAN±STD (%)	RANGE (%)	RANK
DDA	9.00±4.01	4.17-19.15	8
DDP	11.48±2.21	6.94-14.09	11
DMA	11.73±1.97	8.93-16.77	12
DMP	7.42±2.56	3.97-11.31	6
DPA	10.00±3.36	2.18-13.39	9
DPP	10.52±2.51	3.77-14.38	10
VDA	5.85±2.58	1.29-10.52	2
VDP	6.83±2.93	1.79-12.10	4
VMA	6.16±2.30	2.28-12.80	3
VMP	5.79±2.18	1.79-10.52	1
VPA	7.82±4.90	0.79-16.87	7
VPP	7.27±1.38	5.06- 9.82	5
WHOLE MUSCLE	8.32±3.50	0.79-19.15	

b)

MUSCLE PARTS	MEAN±STD (%)	RANGE (%)	RANK
DDA	9.55±1.34	5.95-12.00	3
DDP	9.04±3.62	4.86-17.16	2
DMA	9.64±2.07	5.85-14.38	4
DMP	9.75±2.70	5.56-14.68	5
DPA	11.71±2.30	8.53-17.36	9
DPP	7.83±2.86	3.27-14.09	1
VDA	14.78±3.16	9.72-19.74	12
VDP	12.43±2.67	7.94-16.96	11
VMA	10.93±3.74	6.75-23.12	6
VMP	11.63±1.60	8.23-14.19	8
VPA	11.72±1.98	7.14-16.07	10
VPP	11.62±3.05	7.04-18.45	7
WHOLE MUSCLE	10.89±3.17	3.27-19.74	

parts ranked with respect to the mean mitochondrial area of their fibres (table 12b; fig. 34) showed that, the parts with larger mitochondrial area tended to be significantly different from the parts with smaller area.

The ANOVA test showed that the population of the dorsal region of the female, with the mean mitochondrial area of 9.59±2.78%, was significantly different from that of the ventral region, with the mean of 12.18±3.0%, this indicates that some of the parts of either region are different from all of the parts from of the other region ($p < 0.001$). The dorsal parts with exception of DPA had smaller mitochondrial area than the ventral parts. The medial muscle parts tended to have medium mitochondrial area. The mean and range of mitochondrial area within the muscle of the female were, 10.89±3.17% and 3.27–19.74% respectively (table 13b).

Mitochondria which were located between myofibrils varied in shape being oval, oblong or irregular (fig. 35). Sometimes the myofibrils were surrounded by mitochondria (fig. 36). In some areas pairs of mitochondria and elongated mitochondria were found (fig. 37) and in others the mitochondria were scattered (fig. 38).

The ANOVA test for the mitochondrial area showed that the population of the whole muscle of the male was significantly different that of the female ($p < 0.001$). The ANOVA also showed that the population of the dorsal region was significantly

Fig. 35: Cross section of the muscle fibre, showing the different shapes of mitochondrial profile. X 16,000.

Fig. 36: Cross section of the muscle fibre, showing the mitochondria surrounding the myofibrils. X 16,000.

Fig. 37: Cross section of the muscle fibre, with pairs of mitochondria and large mitochondria with high density. X 16,000

Fig. 38: Cross section of the muscle fibre, showing the scattered mitochondria between the myofibrils. X 16,000.

different from the ventral region within the muscles of both the male and the female ($p < 0.001$), however, with different F ratio ($F=75.15$ for the male and 50.93 for the female). This may suggest that the muscle of the female is less heterogeneous.

The range and STD of mitochondrial area within each individual part of the muscle of both the male and the female (table 13a, b) were great i.e. in each individual part of the muscles, fibres with notably different mitochondrial area existed. The STD and range of mitochondrial area within the muscles showed that, variation of mitochondrial area within the muscle of the male was greater than that of the female. The female had higher mitochondria area than the male. The significant differences between the parts within the muscle of the male and female, the wide range of mitochondrial area within the whole muscles and within each individual part, showed that the muscles were a mixture of fibres with significantly smaller and larger mitochondrial area (table 13a, b).

The parts with lowest and highest mitochondrial number within the muscle of the male were, VDA and DPA with mean mitochondrial numbers per 100 μm^2 of 30.34 ± 9.55 and 48.43 ± 10.17 respectively (table 14a). The ANOVA test for the number of mitochondria showed that the population was not homogeneous and that some muscle parts within the male were significantly different from others ($p < 0.001$). The T-test between individual parts showed that the minority of the

Table 14: Mean number of mitochondria per 100 μm^2 of fibres in the 12 parts of the flexor tibialis muscle of a) male and b) female ranked from the lowest to the highest percentage. Significant difference between pairs was tested at p=0.05 (x) indicates no significant difference, (-) significant difference.

a)

	VDA	VPA	VPP	VMA	VMP	DPP	DDA	DDP	VDP	DMP	DMA	DPA	MEAN±STD
VDA		x	x	-	-	-	-	-	-	-	-	-	30.34± 9.55
VPA	x		x	x	x	x	x	-	-	-	-	-	31.96±16.44
VPP	x	x		x	x	-	-	-	-	-	-	-	34.36± 7.62
VMA	-	x	x		x	x	x	x	-	-	-	-	37.90± 8.32
VMP	-	x	x	x		x	x	x	x	x	x	-	39.81±10.66
DPP	-	x	-	x	x		x	x	x	x	x	-	40.87±11.44
DDA	-	x	-	x	x	x		x	x	x	x	-	41.06±11.72
DDP	-	-	-	x	x	x	x		x	x	x	-	41.73± 9.45
VDP	-	-	-	-	x	x	x	x		x	x	x	44.21±10.16
DMP	-	-	-	-	x	x	x	x	x		x	x	44.69±12.25
DMA	-	-	-	-	x	x	x	x	x	x		x	45.17± 9.01
DPA	-	-	-	-	-	-	-	-	x	x	x		48.43±10.17

b)

	DDP	DDA	DMA	DPP	DPA	VDP	DMP	VDA	VMP	VMA	VPP	VPA	MEAN±STD
DDP		-	-	x	-	-	-	-	-	-	-	-	34.55± 8.90
DDA	-		x	x	x	-	-	-	-	-	-	-	40.00± 6.54
DMA	-	x		x	x	-	-	-	-	-	-	-	40.20± 8.49
DPP	x	x	x		x	x	x	-	-	-	-	-	40.67±11.86
DPA	-	x	x	x		x	x	x	-	-	-	-	44.69±11.11
VDP	-	-	-	x	x		x	x	x	-	-	-	46.61± 8.41
DMP	-	-	-	x	x	x		x	x	-	-	-	47.85±13.58
VDA	-	-	-	-	x	x	x		x	-	-	-	48.52± 9.61
VMP	-	-	-	-	-	x	x	x		x	-	-	51.49± 8.60
VMA	-	-	-	-	-	-	-	-	x		x	x	57.23± 9.44
VPP	-	-	-	-	-	-	-	-	-	x		x	59.14±10.09
VPA	-	-	-	-	-	-	-	-	-	x	x		61.73±10.61

muscle parts were significantly different from each other ($p=0.05$). The muscle parts ranked with respect to the mean mitochondrial number of their fibres (table 14a, fig. 39) showed that, the parts with larger mitochondrial numbers were significantly different from the parts with smaller numbers.

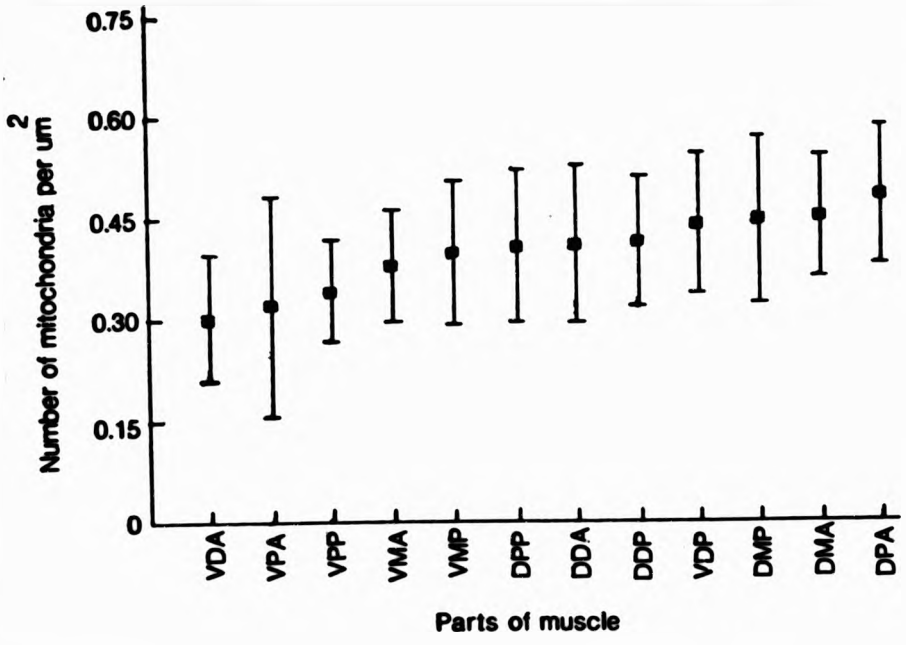
The ANOVA test showed that the population of the dorsal region of the male, with the mean mitochondrial number per 100 μm^2 of 43.66±10.86, was significantly different from that of the ventral region, with mean number of 36.43±11.64, this indicates that some of the parts of either region are different from all of the parts of the other region ($p < 0.001$). The ventral parts of the muscle with the exception of VDP had smaller number of mitochondria than the dorsal parts. The distal and proximal parts tended to have lower, medium and larger number of mitochondria and medial parts tended to have medium and larger number. The mean and range of mitochondrial number per 100 μm^2 of the male were, 40.04±11.80 and 7.66–68.91 respectively (table 15a).

The parts with lowest and highest mitochondrial number within the muscle of the female were, DDP and VPA with mean mitochondrial number per 100 μm^2 of 34.55±8.9 and 61.73±10.61 respectively (table 14b). The ANOVA test for the number of mitochondria showed that the population was not homogeneous and that some muscle parts within the female were significantly different from others ($p < 0.001$). The T-test between individual parts showed that the majority of the

Fig. 39: Showing the muscle parts from the male ranked, according to their mean number of mitochondria per μm^2 of fibres.

Fig. 40: Showing the muscle parts from the female ranked, according to their mean number of mitochondria per μm^2 of fibres.

In the muscle of the male the dorsal parts have a higher mitochondrial number per μm^2 which is opposite of the female where the ventral parts have higher mitochondrial number.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VPP
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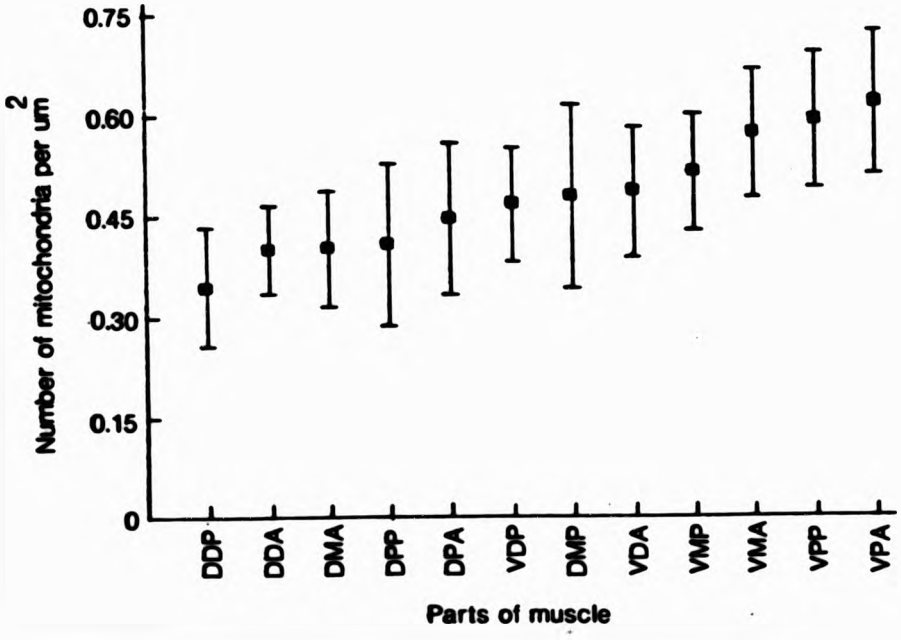
DPA
Parts of muscle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Number of mitochondria per um 2
DDP
DDA
DMA
DPP
DPA
VDP
DMP
VDA
VMP
VMA
VPP
VPA
Parts of muscle

Table 15: Mean, standard deviation and range of number of mitochondria per 100 μm^2 of fibres within the 12 parts and the flexor tibialis muscle of a) male and b) female. The numbers in final column shows the rank position for mitochondrial number (mean) of the 12 muscle parts. Number (1) indicates the smallest and number (12) the largest mitochondrial number.

a)

MUSCLE PARTS	MEAN±STD	RANGE	RANK
DDA	41.06±11.72	26.80-63.16	7
DDP	41.73± 9.45	26.80-65.08	8
DMA	45.17± 9.01	32.54-61.25	11
DMP	44.69±12.25	24.88-65.08	10
DPA	48.43±10.17	30.62-68.91	12
DPP	40.87±11.44	13.40-63.16	6
VDA	30.34± 9.55	11.48-47.85	1
VDP	44.21±10.16	24.88-65.08	9
VMA	37.90± 8.32	24.88-51.68	4
VMP	39.81±10.66	17.23-53.59	5
VPA	31.96±16.44	7.66-59.34	2
VPP	34.36± 7.62	21.05-47.85	3
WHOLE MUSCLE	40.04±11.80	7.66-68.91	

b)

MUSCLE PARTS	MEAN±STD	RANGE	RANK
DDA	40.00± 6.54	28.71-53.59	2
DDP	34.55± 8.90	15.31-47.85	1
DMA	40.20± 8.49	28.71-63.16	3
DMP	47.85±13.58	26.80-72.73	7
DPA	44.69±11.11	30.62-74.65	5
DPP	40.67±11.86	24.88-63.16	4
VDA	48.52± 9.61	30.62-66.99	8
VDP	46.61± 8.41	28.71-65.08	6
VMA	57.23± 9.44	40.20-74.65	10
VMP	51.49± 8.60	34.45-65.08	9
VPA	61.73±10.61	42.11-80.39	12
VPP	59.14±10.09	42.11-78.48	11
WHOLE MUSCLE	47.72±12.63	15.31-80.39	

muscle parts of the female were significantly different from each other ($p=0.05$). The muscle parts ranked with regard to the mean mitochondrial number of their fibres (table 14b, fig. 40) showed that only the parts which were juxtaposed within the ranking were not significantly different from each other.

The ANOVA test showed that the population of the dorsal region of the female, with the mean mitochondrial number per 100 μm^2 of 41.33±10.95, was significantly different from that of the ventral region, with the mean number of 54.12±0.85, this indicates that some of the parts of either region are different from all of the parts of the other region ($p < 0.001$). The dorsal parts with exception of DMP had smaller numbers of mitochondria than the ventral parts. The distal parts tended to have lower and medium number of mitochondria, the medial and proximal tended to have lower, medium and larger number. The mean and range of mitochondrial number per μm^2 of the female were, 47.72±12.63 and 15.31–80.39 respectively (table 15b).

The ANOVA test for the mitochondrial number showed that the population of the whole muscle of the male was significantly different from that of the female ($p < 0.001$). The ANOVA also showed that the population of the dorsal region was significantly different from that of the ventral region within the muscles of both the male and the female ($p < 0.001$), however, with different F ratio ($F=25.14$ for the male and $F=95.91$ for the female). This may suggest that the muscle of

the male is less heterogeneous.

The range and STD showed that the variation between the mitochondrial number of fibres within the muscle of the female was greater than that of the male. In some fibres mitochondria were of high density and surrounded the myofibrils (fig. 37), however in other the mitochondria were scattered (fig. 38).

The significant differences between parts, the great range and STD within the whole muscle of the male and the female and within each individual part showed that, the muscles were a mixture of fibres with significantly lower and higher mitochondrial number (table 15a, b).

The parts with the smallest and largest mitochondria within the muscle of the male were, VMP which had mitochondria with average profile area of 0.15±0.04 μm^2 and DDP with average mitochondrial profile area of 0.29±0.07 μm^2 (table 16a). The ANOVA test for the size of mitochondria showed that the population was not homogeneous and that some muscle parts within the male were significantly different from others ($p < 0.001$). The T-test between individual parts showed that most parts within the muscle were significantly different from each other ($p=0.05$). The muscle parts ranked with respect to the average area of mitochondrial profile of their fibres (table 16a, fig. 41) showed that, the muscle parts with smaller mitochondria were significantly different from the parts with larger ones, also the parts with medium mitochondria were

Table 16: Average size of mitochondria (μm^2) of fibres in the 12 parts of the flexor tibialis muscle of a) male and b) female ranked from the smallest to the largest average sizes. Significant difference between pairs was tested at p=0.05. (x) indicates no significant difference, (-) significant difference.

a)

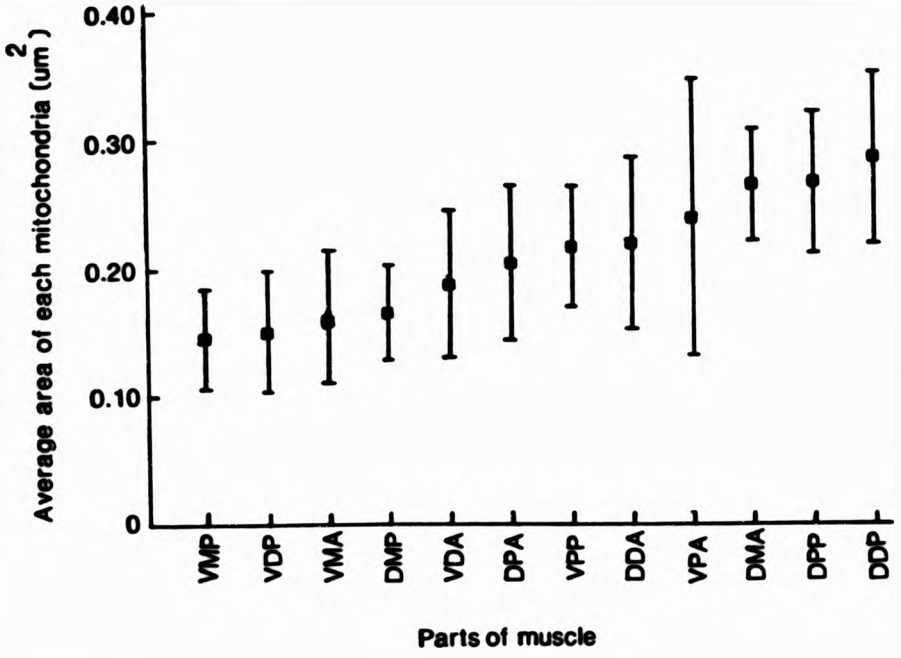
	VMP	VDP	VMA	DMP	VDA	DPA	VPP	DDA	VPA	DMA	DPP	DDP	MEAN±STD (μm^2)
VMP		x	x	x	–	–	–	–	–	–	–	–	0.15±0.04
VDP	x		x	x	–	–	–	–	–	–	–	–	0.15±0.05
VMA	x	x		x	x	–	–	–	–	–	–	–	0.16±0.05
DMP	x	x	x		x	–	–	–	–	–	–	–	0.17±0.04
VDA	–	–	x	x		x	x	x	x	–	–	–	0.19±0.06
DPA	–	–	–	–	x		x	x	x	–	–	–	0.20±0.06
VPP	–	–	–	–	x	x		x	x	–	–	–	0.22±0.05
DDA	–	–	–	–	x	x	x		x	–	–	–	0.22±0.07
VPA	–	–	–	–	x	x	x	x		x	x	x	0.24±0.11
DMA	–	–	–	–	–	–	–	–	x		x	x	0.26±0.04
DPP	–	–	–	–	–	–	–	–	x	x		x	0.27±0.05
DDP	–	–	–	–	–	–	–	–	x	x	x		0.29±0.07

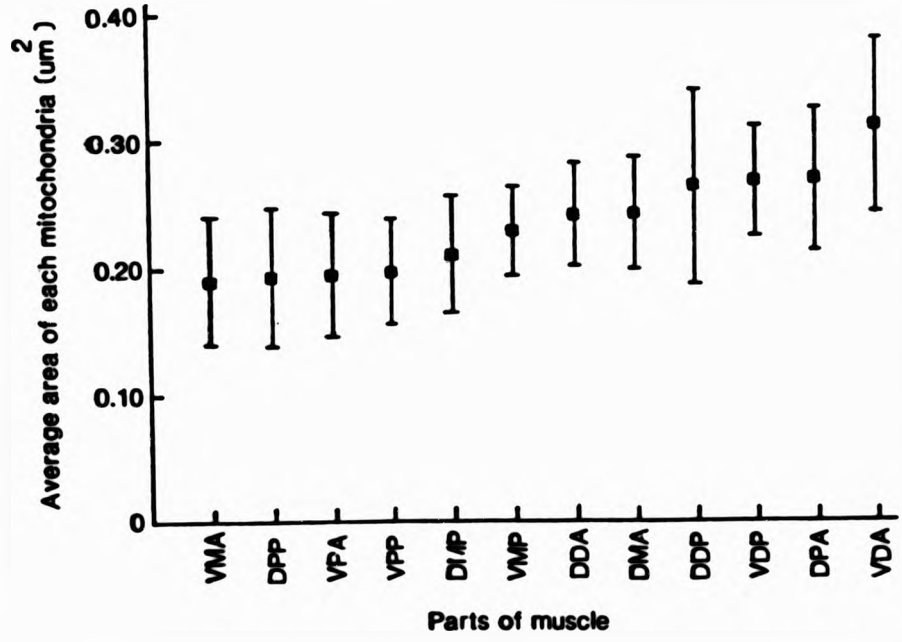
b)

	VMA	DPP	VPA	VPP	DMP	VMP	DDA	DMA	DDP	VDP	DPA	VDA	MEAN±STD (μm^2)
VMA		x	x	x	x	–	–	–	–	–	–	–	0.19±0.05
DPP	x		x	x	x	–	–	–	–	–	–	–	0.19±0.05
VPA	x	x		x	x	–	–	–	–	–	–	–	0.19±0.05
VPP	x	x	x		x	–	–	–	–	–	–	–	0.20±0.04
DMP	x	x	x	x		x	–	–	–	–	–	–	0.21±0.05
VMP	–	–	–	–	x		x	x	x	–	–	–	0.23±0.03
DDA	–	–	–	–	–	x		x	x	x	x	–	0.24±0.04
DMA	–	–	–	–	–	x	x		x	x	x	–	0.24±0.04
DDP	–	–	–	–	–	x	x	x		x	x	–	0.26±0.07
VDP	–	–	–	–	–	–	x	x	x		x	–	0.27±0.04
DPA	–	–	–	–	–	–	x	x	x	x		–	0.27±0.06
VDA	–	–	–	–	–	–	–	–	–	–	–		0.31±0.07

Fig. 41: Showing the muscle parts from the male ranked, according to the average size of their mitochondrial profiles (μm^2) of fibres.

Fig. 42: Showing the muscle parts from the female ranked, according to the average size of their mitochondrial profiles (μm^2) of fibres.


Average area of each mitochondria (um 2)
0.40
0.30
0.20
0.10
0
VMP
VDP
VMA
DMP
VDA
DPA
VPP
DDA
VPA
DMA
DPP
DDP
Parts of muscle


Average area of each mitochondria (um 2)
0.40
0.30
0.20
0.10
0
VMA
DPP
VPA
VPP
DMP
VMP
DDA
DMA
DDP
VDP
DPA
VDA
Parts of muscle

significantly different from both parts with larger and smaller mitochondria.

The ANOVA test showed that the population of the dorsal region of the male, with the mean mitochondrial size of 0.23±0.07 μm^2, was significantly different from that of the ventral region, with the mean of 0.18±0.07 μm^2, this indicates that some of the parts of either region are different from all of the parts of the other region ($p < 0.001$). Most dorsal parts have larger mitochondria than the ventral parts. The proximal parts tended to have medium and larger size of mitochondria and the medial parts tended to have smaller size. Mean and range of area of mitochondrial profile within the muscle of the male were, 0.21 ±0.07 μm^2 and 0.06-0.51 μm^2 respectively (table 17a).

The parts with smallest and largest mitochondria within the muscle of the female were, VMA which had mitochondria with average profile area of 0.19±0.05 μm^2 and VDA with the profile average area of 0.31±0.07 um^2 respectively (table 16b). The ANOVA test for the size of mitochondria showed that the population was not homogeneous and that some muscle parts within the female were significantly different from others ($p < 0.001$). The T-test between individual parts showed that the majority of the parts within the muscle were significantly different from each other ($p=0.05$). The muscle parts ranked with regard to the average area of mitochondrial profile of their fibres (table 16b, fig. 42) showed that, the parts with

Table 17: Mean, standard deviation and range of each mitochondrial profile size (μm^2) of fibres within the 12 parts and the flexor tibialis muscle of a) male and b) female. The numbers in final column shows the rank position for mitochondrial size (mean) of the 12 muscle parts. Number (1) indicates the smallest and number (12) the largest mitochondrial size.

a)

MUSCLE PARTS	MEAN±STD (μm^2)	RANGE (μm^2)	RANK
DDA	0.22±0.07	0.12-0.34	8
DDP	0.29±0.07	0.11-0.40	12
DMA	0.27±0.04	0.19-0.34	10
DMP	0.17±0.04	0.09-0.26	4
DPA	0.20±0.06	0.07-0.30	6
DPP	0.27±0.06	0.17-0.40	11
VDA	0.19±0.06	0.08-0.29	5
VDP	0.15±0.05	0.06-0.21	2
VMA	0.16±0.05	0.09-0.26	3
VMP	0.15±0.04	0.09-0.21	1
VPA	0.24±0.11	0.07-0.51	9
VPP	0.22±0.05	0.16-0.39	7
WHOLE MUSCLE	0.21±0.07	0.06-0.51	

b)

MUSCLE PARTS	MEAN±STD (μm^2)	RANGE (μm^2)	RANK
DDA	0.24±0.04	0.18-0.31	7
DDP	0.26±0.08	0.14-0.43	9
DMA	0.24±0.04	0.14-0.31	8
DMP	0.21±0.05	0.14-0.30	5
DPA	0.27±0.06	0.19-0.43	11
DPP	0.19±0.05	0.12-0.31	2
VDA	0.31±0.07	0.21-0.43	12
VDP	0.27±0.04	0.19-0.37	10
VMA	0.19±0.05	0.11-0.35	1
VMP	0.23±0.03	0.15-0.30	6
VPA	0.20±0.05	0.14-0.33	3
VPP	0.20±0.04	0.14-0.29	4
WHOLE MUSCLE	0.23±0.06	0.11-0.43	

smaller mitochondria tended to be significantly different from the parts with medium and larger mitochondria.

The ANOVA test showed that the population of the dorsal region of the female, with the mean mitochondrial size of 0.24±0.06 μm^2, was not significantly different from the ventral region, with the mean of 0.23±0.07 μm^2, this indicates that none of the parts of either region is different from all of the parts of the other region ($p=0.479$). Whereas the T-test showed that some of the dorsal parts were significantly different from some of ventral parts ($p=0.05$). Most ventral parts tended to have smaller mitochondria than dorsal parts. Proximal and medial parts tended to have smaller mitochondria and distal parts larger mitochondria. Mean and range mitochondrial profile area within the muscle of the female were, 0.23±0.06 μm^2 and 0.11-0.43 μm^2 respectively (table 17b).

The ANOVA for size of mitochondria showed that the population of the whole muscle of the male was significantly different from that of the female ($p < 0.001$). The ANOVA also showed that the population of the dorsal region was significantly different from that of the ventral region within the muscle from the male ($p < 0.001$), but the population of the dorsal region was not significantly different from that of the ventral region within the female ($p=0.479$). This suggests that the muscle of the female is less heterogeneous.

The range and STD of mitochondrial size of the muscle showed

that, variation between the mitochondrial sizes within the muscle of the male was greater than that in the muscle of the female. In each individual part, mitochondria with notably different sizes existed. Some mitochondria were larger, 2 or 3 of them were surrounding the myofibril (fig. 37) and others were smaller (fig. 43).

The significant differences between mean mitochondrial sizes of the most muscle parts, wide range and STD in each individual part and within the whole muscle of the male and female (table 17a, b) showed that, the muscles were a mixture of fibres with significantly smaller and larger mitochondria.

3.3.7. The area of sarcoplasmic reticulum

The parts with the lowest and highest area of sarcoplasmic reticulum (SR) within the muscle of the male were, DDA with mean percentage area of 8.08±2.4% and VPP with the mean area of 18.96±3.27% (table 18a). The ANOVA test for the area of SR showed that the population was not homogeneous and that some muscle parts within the male were significantly different from others ($p < 0.001$). The T-test between individual parts showed that most parts of the muscle from the male were significantly different from each other ($p=0.05$). The muscle parts ranked with regard to the mean percentage area of SR from their fibres (table 18a; fig. 44) showed that the parts which were not significantly different tended to have the medium area of SR.

Fig. 43: Cross section of the muscle fibre, showing the very small mitochondria. X 16,000.

Table 18: Mean percentage area of sarcoplasmic reticulum of fibres in the 12 parts of the flexor tibialis muscle of a) male and b) female ranked from the highest to the lowest percentage. Significant difference between pairs was tested at p=0.05. (x) indicates no significant difference, (-) significant difference.

a)

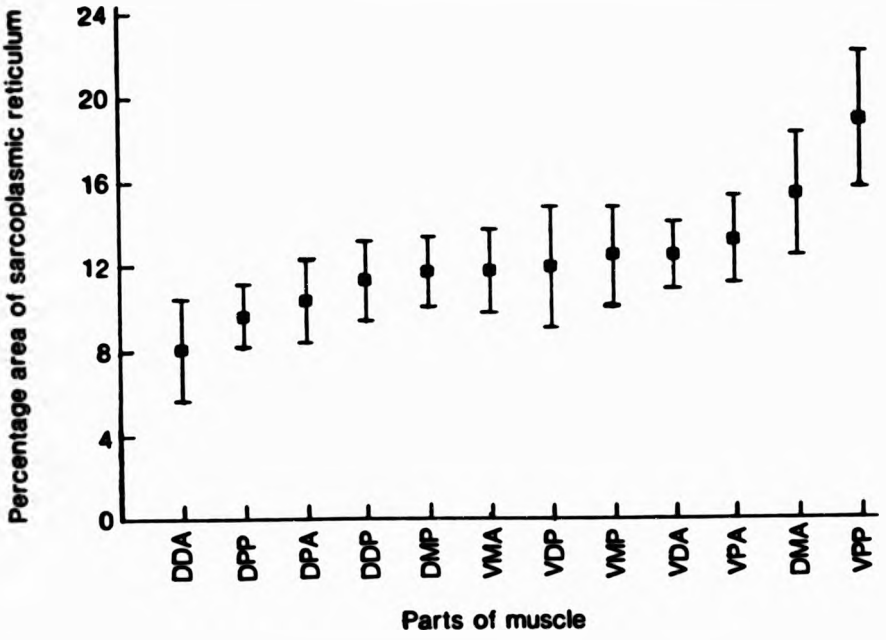
	VPP	DMA	VPA	VDA	VMP	VDP	VMA	DMP	DDP	DPA	DPP	DDA	MEAN±STD (%)
VPP		-	-	-	-	-	-	-	-	-	-	-	18.96±3.27
DMA	-		-	-	-	-	-	-	-	-	-	-	15.37±2.91
VPA	-	-		x	x	x	-	-	-	-	-	-	13.25±2.06
VDA	-	-	x		x	x	x	x	-	-	-	-	12.52±1.58
VMP	-	-	x	x		x	x	x	x	-	-	-	12.48±2.34
VDP	-	-	x	x	x		x	x	x	x	-	-	11.95±2.85
VMA	-	-	-	x	x	x		x	x	-	-	-	11.76±1.95
DMP	-	-	-	x	x	x	x		x	-	-	-	11.74±1.64
DDP	-	-	-	-	x	x	x	x		x	-	-	11.32±1.92
DPA	-	-	-	-	-	x	-	-	x		x	-	10.38±2.00
DPP	-	-	-	-	-	-	-	-	-	x		-	9.66±1.48
DDA	-	-	-	-	-	-	-	-	-	-	-		8.08±2.40

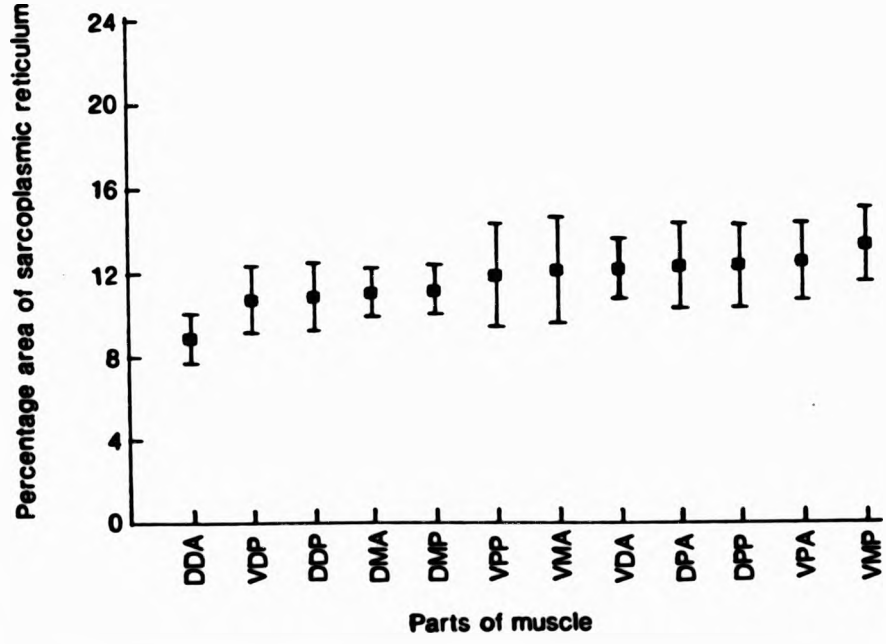
b)

	VMP	VPA	DPP	DPA	VDA	VMA	VPP	DMP	DMA	DDP	VDP	DDA	MEAN±STD (%)
VMP		x	x	x	-	x	-	-	-	-	-	-	13.31±1.74
VPA	x		x	x	x	x	x	-	-	-	-	-	12.52±1.85
DPP	x	x		x	x	x	x	-	-	-	-	-	12.31±1.95
DPA	x	x	x		x	x	x	-	-	-	-	-	12.30±2.05
VDA	-	x	x	x		x	x	-	-	-	-	-	12.19±1.43
VMA	x	x	x	x	x		x	x	x	x	-	-	12.08±2.53
VPP	-	x	x	x	x	x		x	x	x	x	-	11.87±2.48
DMP	-	-	-	-	-	x	x		x	x	x	-	11.22±1.16
DMA	-	-	-	-	-	x	x	x		x	x	-	11.05±1.16
DDP	-	-	-	-	-	x	x	x	x		x	-	10.88±1.62
VDP	-	-	-	-	-	-	x	x	x	x		-	10.71±1.59
DDA	-	-	-	-	-	-	-	-	-	-	-		8.87±1.13

Fig 44: Showing the muscle parts from the male ranked, according to their mean percentage area of sarcoplasmic reticulum of fibres.

Fig 45: Showing the muscle parts from the female ranked, according to their mean percentage area of sarcoplasmic reticulum of fibres.


Percentage area of sarcoplasmic reticulum
24
20
16
12
8
4
0
DDA
DPP
DPA
DDP
DMP
VMA
VDP
VMP
VDA
VPA
DMA
VPP
Parts of muscle


Percentage area of sarcoplasmic reticulum
24
20
16
12
8
4
0
DDA
VDP
DDP
DMA
DMP
VPP
VMA
VDA
DPA
DPP
VPA
VMP
Parts of muscle

The ANOVA test showed that the population of the dorsal region of the male, with the mean SR area of 11.09±3.06%, was significantly different from that of the ventral region, with the mean of 13.49±3.44%, this indicates that some of the parts of either region are different from all of the parts of the other region ($p < 0.001$). The dorsal parts, with exception of DMA which had the second highest area of SR, had a lower area than ventral ones. The distal parts tended to have smaller and medium areas of SR, the medial parts have medium and larger areas of SR and the proximal have smaller and larger areas. The mean and range of the area of SR within the muscle of the male were, 12.29±3.47% and 3.87–25.30% (table 19a).

The parts with the lowest and highest area of SR within the muscle of the female were, DDA and VMP with the mean percentage area of 8.87±1.13% and 13.31±1.74% respectively (table 18b). The ANOVA test for the area of SR showed that the population was not homogeneous and that some muscle parts within the female were significantly different from others ($p < 0.001$). The T-test between individual parts showed that there were significant differences between about 50% of parts from the muscle ($p=0.05$). The muscle parts ranked with respect to the mean percentage area of SR from their fibres (table 18b; fig. 45) showed that, the parts with lower area were significantly different from those with higher area.

The ANOVA test showed that the population of the dorsal region of the female, with the mean SR area of 11.10±1.91%, was

Table 19: Mean, standard deviation and range of percentage area of sarcoplasmic reticulum of fibres within the 12 parts and the flexor tibialis muscle of a) male and b) female. The numbers in final column shows the rank position for sarcoplasmic reticulum area (mean) of the 12 muscle parts. Number (1) indicates the highest and number (12) the lowest sarcoplasmic reticulum area.

a)

MUSCLE PARTS	MEAN±STD (%)	RANGE (%)	RANK
DDA	8.08±2.40	3.87-12.90	12
DDP	11.32±1.92	7.44-14.48	9
DMA	15.37±2.91	9.72-19.64	2
DMP	11.74±1.64	7.64-14.19	8
DPA	10.38±1.99	6.94-15.77	10
DPP	9.66±1.48	6.45-12.10	11
VDA	12.52±1.58	10.12-15.68	4
VDP	11.95±2.85	8.14-18.85	6
VMA	11.76±1.95	7.24-14.38	7
VMP	12.48±2.34	9.13-18.45	5
VPA	13.25±2.06	9.82-17.56	3
VPP	18.96±3.27	12.90-25.30	1
WHOLE MUSCLE	12.29±3.47	3.87-25.30	

b)

MUSCLE PARTS	MEAN±STD (%)	RANGE (%)	RANK
DDA	8.87±1.13	6.75-10.81	12
DDP	10.88±1.62	8.14-13.50	10
DMA	11.05±1.16	9.03-13.39	9
DMP	11.22±1.16	8.93-13.29	8
DPA	12.30±2.05	9.72-19.05	4
DPP	12.31±1.95	7.74-15.68	3
VDA	12.19±1.43	8.73-14.48	5
VDP	10.71±1.59	7.94-13.50	11
VMA	12.08±2.53	7.14-17.06	6
VMP	13.31±1.74	9.62-15.87	1
VPA	12.52±1.85	8.53-15.28	2
VPP	11.87±2.48	7.74-16.37	7
WHOLE MUSCLE	11.61±2.06	6.75-19.05	

significantly different from that of the ventral region, with the mean of 12.11±2.09%, this indicates that some of the parts of either region are different from all of the parts of the other region ($p < 0.001$). Most dorsal parts had lower area of SR than ventral parts. The proximal parts tended to have larger areas of SR, the medial parts tended to have medium and larger areas and distal parts tended to have smaller areas of SR. The mean and range of the area of SR within the muscle of the female were 11.61±2.06% and 6.75–19.05% respectively (table 19b).

In some fibres the small polygonal myofibrils were surrounded by a well developed SR with several dyads (fig. 46). In others large strap like myofibrils were surrounded by the SR i.e. these muscle fibres had less area of SR and dyads per unit area (fig. 47).

The ANOVA test for areas of SR showed that the population of the whole muscle of the male was significantly different from that of the female ($p=0.009$). The ANOVA also showed that the population of the dorsal region was significantly different from that of the ventral region within the muscles of both the male and the female ($p < 0.001$), but with different F ratio ($F=46.09$ for the male and $F=17.75$ for the female). This may suggest that the muscle of the female is less heterogeneous.

The range and STD of areas of SR within the muscles showed that, variation between the area of SR of fibres within the

Fig. 46: Cross section of the muscle fibre, with small polygonal myofibrils surrounded by abundant sarcoplasmic reticulum and dyads. This structure is typical of fast fibres. X 16,000.

Fig. 47: Cross section of the muscle fibre, with large strap like myofibrils surrounded by sparse sarcoplasmic reticulum and few dyads. This structure is typical of slow fibres. X 16,000

muscle of the male was greater than that of the female. The muscle parts of the male were with relatively higher mean percentage area of SR than the female (table 19a, b). The significant differences between the most parts within the muscles, the wide range of the area of SR within the whole muscles and within each individual part showed that, the muscles were a mixture of fibres with significantly lower and higher areas of SR.

The ANOVA test for all ultrastructural parameters mentioned above showed that the muscles of both the male and female were heterogeneous (table 20). The ANOVA showed that some parts within the muscles of both the male and the female were highly significantly different from others (for all the parameters $P<0.001$). The ANOVA also showed that the population of the dorsal region was significantly different from that of the ventral region, within the muscle of the male (with exception of thick filaments number) and within the female (with exception of thin filaments and mitochondrial number) for all parameters. The T-test for all parameters showed that differences between different parts within the muscles of both male and the female were high ($p=0.05$). The majority of parts within the muscles were significantly different from each other for most criteria (with the exception of mitochondrial number within the male and area of SR within the female).

The ANOVA test for all the ultrastructural criteria (with the exception of thin to thick filaments ratios, $p=0.465$) showed

Table 20: The Analysis of Variance results for all ultrastructural criteria showing; a) the differences between the 12 parts within the muscles, the difference between dorsal and ventral parts (D-V) within the muscles of the male and the female, b) the difference between the population within the male and that of the female (M & F). DF =degree of freedom, F =F ratio and p =level of probability.

For all parameters N=20 except for sarcomere length where N=100.

a)

		MALE			FEMALE		
Criteria		DF	F	P	DF	F	P
Sarcomere length	12 parts	11	112.48	<0.001	11	33.38	<0.001
	D-V	1	289.42	<0.001	1	9.04	0.003
Fibre area	12 parts	11	34.75	<0.001	11	17.93	<0.001
	D-V	1	479.14	<0.001	1	7.41	0.007
Ratio	12 parts	11	11.87	<0.001	11	13.07	<0.001
	D-V	1	42.01	<0.001	1	7.68	0.006
Thick Fil.No	12 parts	11	16.47	<0.001	11	19.24	<0.001
	D-V	1	2.64	0.105	1	11.58	0.001
Thin Fil.No	12 parts	11	13.25	<0.001	11	6.83	<0.001
	D-V	1	31.90	<0.001	1	0.21	0.644
Mito area	12 parts	11	11.17	<0.001	11	9.40	<0.001
	D-V	1	75.15	<0.001	1	50.93	<0.001
Mito No	12 parts	11	5.25	<0.001	11	14.42	<0.001
	D-V	1	25.14	<0.001	1	95.91	<0.001
Mito size	12 parts	11	12.81	<0.001	11	11.51	<0.001
	D-V	1	35.11	<0.001	1	0.50	0.479
SR area	12 parts	11	30.11	<0.001	11	8.39	<0.001
	D-V	1	46.09	<0.001	1	17.75	<0.001

b)

Criteria	DF	F	P
Sarcomere length	1	23.99	<0.001
Fibre area	1	21.93	<0.001
Ratio	1	0.53	0.465
Thick Fil. No	1	14.27	<0.001
Thin Fil. No	1	28.32	<0.001
Mito area	1	70.75	<0.001
Mito No	1	47.38	<0.001
Mito size	1	15.87	<0.001
SR area	1	6.80	0.009

that the differences between the male and the female were highly significant ((table 20)

The ANOVA test showed either similarity between the levels of probability for differences between the dorsal and ventral regions within the female and those within the male or the levels of probability for the female was higher (with exception of thick filament number in which the level in the male was higher). This may appear that the muscle of the female is less heterogenous than the male (table 20).

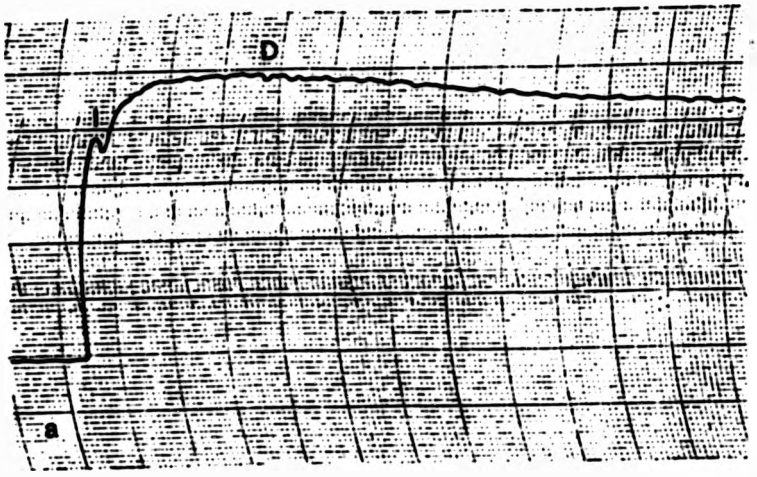
The great range and STD for all the ultrastructural criteria also suggest that the muscles of both the male and female were a mixture of fibres with different ultrastructural properties. The range and STD of the parameters (with the exception of number of mitochondria) showed that, the variation between muscle fibres within the male was greater than that in the female. This may also suggest that the muscle of the female is less heterogeneous.

3.4. MUSCLE PHYSIOLOGY

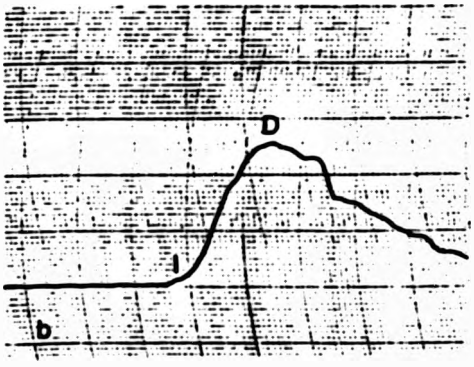
The primary response to an electrical stimulus applied to the thorax, was a contraction of the flexor tibialis muscle which can be divided into 2 phases (fig. 48). The initial, twitch, phase was a rapid reflex contraction of constant amplitude with duration of 70 msec to 130 msec. This was followed by a slower contraction (delayed contraction) which was much longer lasting, generating much more power output and more variable than the initial one. This variable delayed contraction is therefore more influenced by sensory input and central control than the initial contraction. During the delayed contraction the muscle may remain in contraction for more than 30 sec. The amplitude of the delayed contraction was higher than that of the initial phase i.e. the muscle did more work and produced higher tension. Fig. 48 also shows that the muscle does not fatigue rapidly at low loads, but the rate of fatigue increases greatly at high loads. The muscle contractions under conditions of low and high loads showed that when the muscle sustained a low load of 278.4 g the initial, twitch, contraction of the muscle was very high, but the contraction decreased to almost zero with high load of 2088 g. Whereas when the load sustained by the muscle was increased from low load of 278.4 to 2088 g the delayed contraction only decreased about 50% (fig. 48). When the muscle sustained a low load of 278.4 g the delayed contraction only diminished 12% after 5 sec and did not decrease to zero until more than 30 sec. When

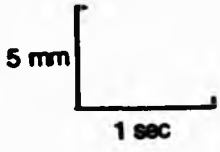
Fig. 48: a, Shows the form of the isotonic contraction of the muscle in an intact E. calcarata under conditions of low and high load. When the muscle sustains a load of 278.4 g, the fast initial contraction (I) is followed by the delayed contraction (D), in which the muscle remains in contraction for a relatively long time. b, shows that when the muscle sustains a load of 2088 g the initial contraction (I) is almost zero and the delayed contraction diminishes to zero after a relatively short time.

278.4 g



2088 g





the muscle sustained a heavy load of 2088 g the delayed contraction diminished to zero after 2.5 sec (fig. 48).

With increases in sustained load by the muscle the initial contraction decreased rapidly and depressed considerably by high load but the delayed contraction decreased much more slowly i.e. the delayed contraction was much stronger than the initial one (fig. 49). The amplitude of the delayed contraction was higher than initial one for all loads sustained by the muscle i.e. the ratio of amplitude of the delayed contraction to that of the initial one was high (fig. 49).

When the insect was stimulated repeatedly, at 1 Hz for 1 min every 6 min, the muscle produced a series of 'initial' contractions or twitches with a maximum tension of 2500 g (fig. 50). The tension produced within the first burst diminished about 31% of maximum tension, to 1725 g, after 18 sec. Then the tension remained relatively constant until the end of the burst (fig. 50).

The changes in tension within individual bursts showed that from the 3rd burst the tension diminished gradually throughout each burst and more strongly than in the first two bursts e.g. by 41% of maximum tension, to 1400 g, within 3rd burst, by 51% of the maximum tension, to 940 g, within the 7th burst and by 41% of the maximum tension, to 885 g, within the 10th burst (fig. 50).

Fig. 49: Showing the differential effect of different loads on the initial and delayed Isotonic contractions of the metathoracic flexor tibialis muscle in an intact male E. calcarata. The figure also shows the relative amplitude during delayed contraction to the initial contraction with increases in load.

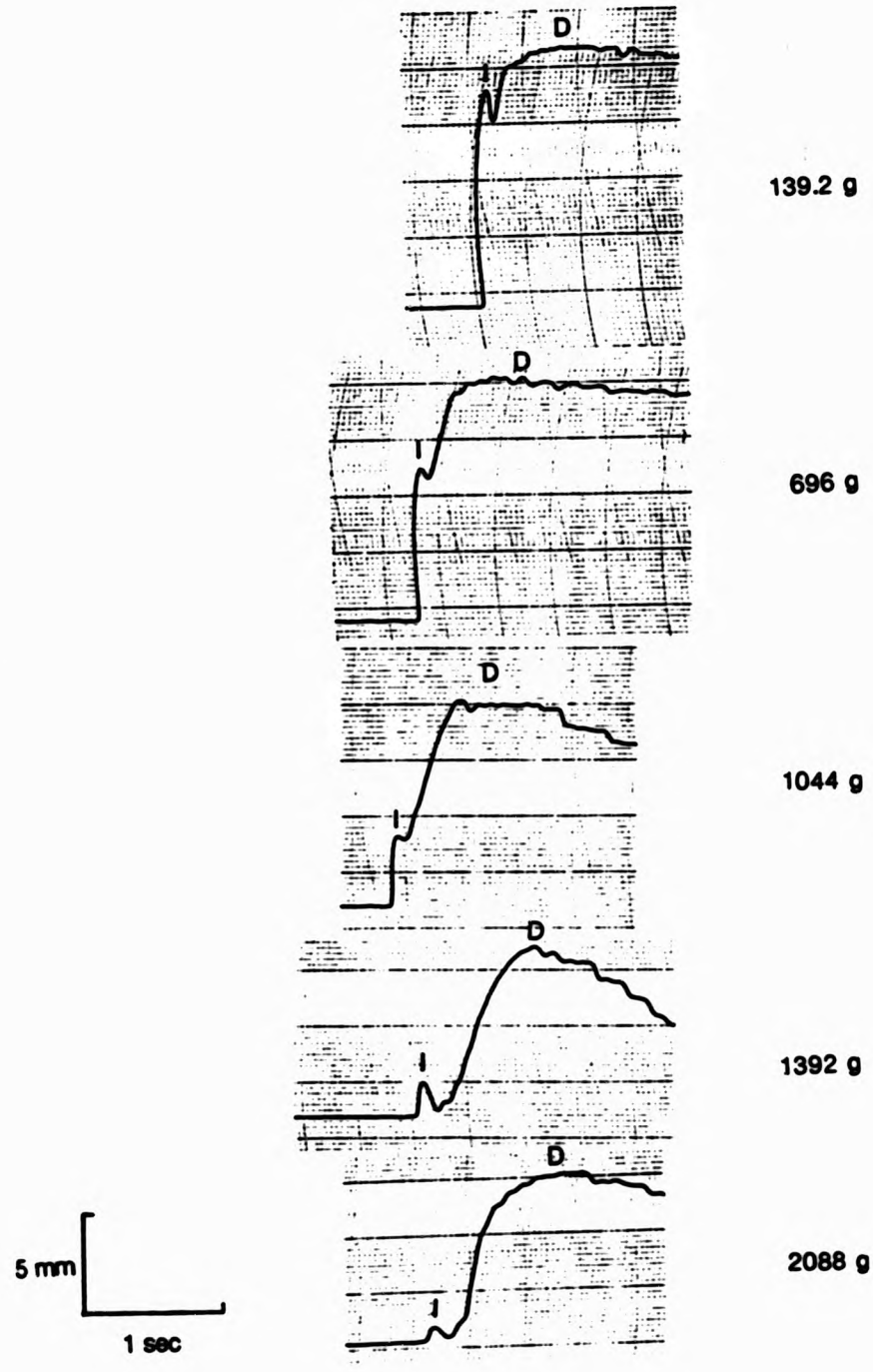
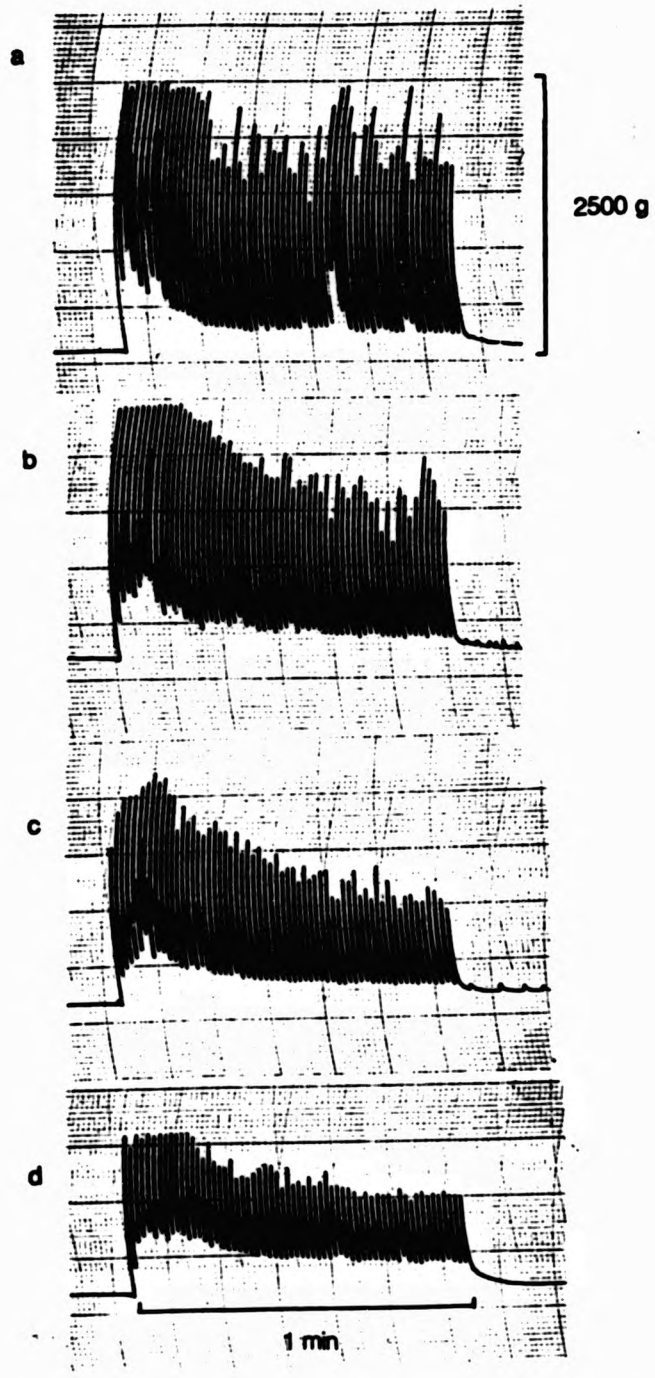

D
I
139.2 g
D
I
696 g
D
I
1044 g
D
I
1392 g
D
I
2088 g
5 mm
1 sec

Fig. 50: Isometric tension produced by the metathoracic flexor tibialis muscle in an intact male E. calcarata. in response to electrical stimulation at 1 Hz for 1 min repeated every 6 min. The traces exhibit the fatigue of the muscle over a 1 hr period. a). Shows the pattern of tension produced within the 1st burst; b). shows 3rd burst, after 18 min; c). shows 7th burst, after 42 min; d). shows the pattern of tension produced within the 10th burst, after 60 min.


a
2500 g
b
c
d
1 min

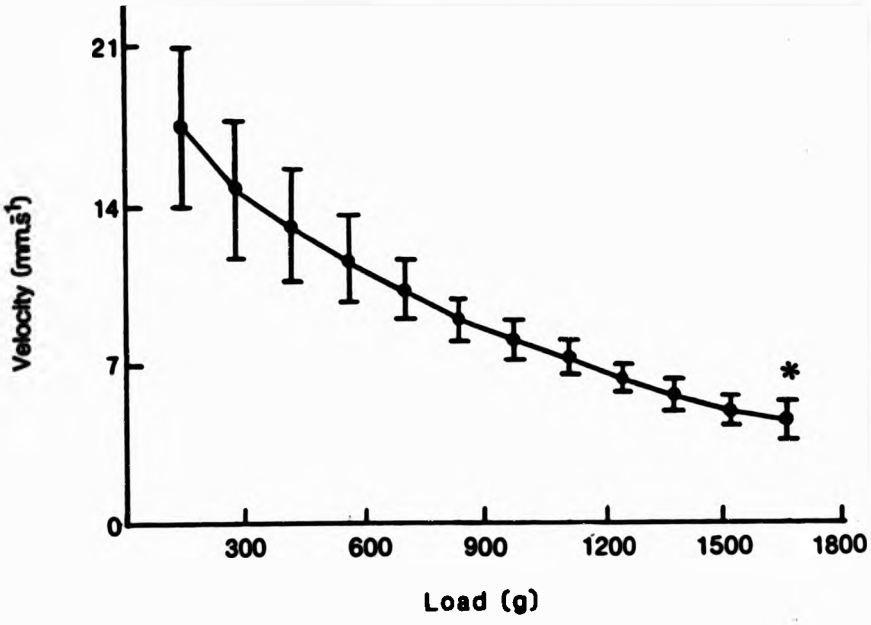
The changes between bursts showed that the initial tension of each burst was similar for the 1st to 3rd burst. The initial tension diminished from the 4th burst onwards, after 26 min continued stimulation (fig. 50). The initial tension diminished for the 7th burst by 23%, to 1930 g, and for the 10th burst by 40%, to 1500 g, of the maximum tension. The terminal tension produced diminished in subsequent bursts, the decreases became greater than that of the initial tension of the bursts. The tension at end of the 3rd burst diminished by 33% of the maximum tension, to 1400 g, for 7th burst by 63%, to 940 g, and at the end of the 10th burst by 65% of the maximum tension, to 885 g. The terminal tension of 10th burst, 885 g, was about 51% of the terminal tension of the 1st burst, 1725 g (fig. 50). The figure also shows that with repetition of stimulation over 1 h the overall decrease in the initial tension was about 40%. This suggests that although the muscle fatigues slightly within each burst and between bursts with time of stimulation, the muscle fatigues quite slowly over an extended period. This also suggests that some of the muscle fibres fatigue more rapidly and that muscle contains a range of physiological fibre types i.e. fibres which contract rapidly and fatigue quickly and fibres which contract less rapidly but exhibit greater fatigue resistance.

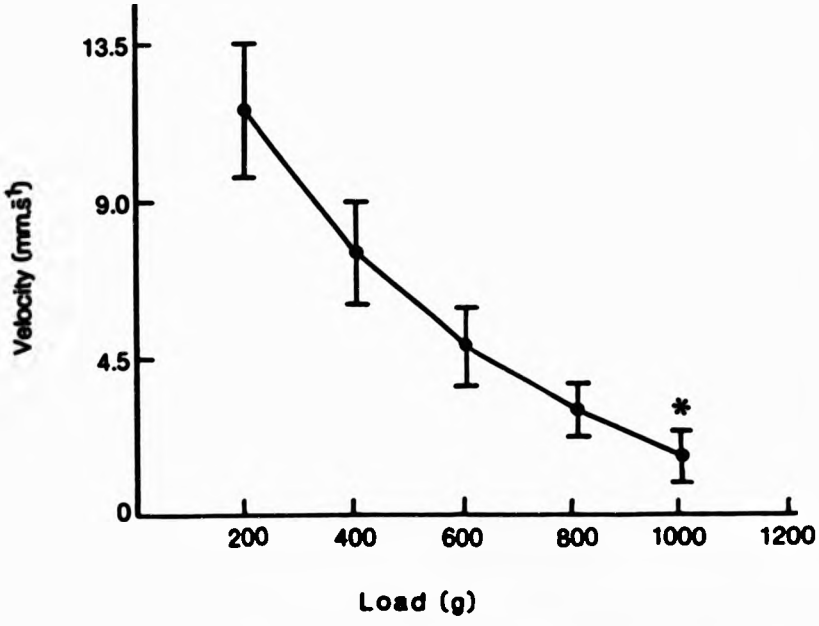
3.4.1. Velocity of initial contraction

As the load was increased the velocity of the muscle decreased (fig. 51), moreover it continued to decrease until it reached zero with very heavy loads. The maximum velocity measured for

Fig. 51: Showing the correlation between the velocity (mean±STD) and increased load in isotonic contraction for the muscle of the males. As the load sustained by the muscle increased, the velocity decreased. In all tests N=25 except for 1670.4 g (*) where N=15.

Fig. 52: Showing the correlation between the velocity (mean± STD) and increased load in isotonic contraction for the muscle of the females. As the load sustained by the muscle increased, the velocity decreased. In all tests N=25 except for 1018 g (*) where N=20.


21
14
7
0
Velocity (mms⁻¹)
300
600
900
1200
1500
1800
Load (g)
*


13.5
9.0
4.5
0
Velocity (mms⁻¹)
200
400
600
800
1000
1200
Load (g)
*

the muscle from the male was 17.37±3.46 $mm.s^{-1}$, with a load of 139.2 g. The minimum velocity measured for the muscle was 4.42±0.9 $mm.s^{-1}$, when the load was 1670.4 g i.e. the heaviest load that most muscles sustained (table 21). Extrapolation of the load-velocity curve showed that, the maximum velocity of the unloaded muscle was about 22.5 $mm.s^{-1}$ (fig. 51). The muscle velocity of the male reaches zero at a maximum load of 2500 g, estimated by extrapolation of the load-velocity curve. As table 21 shows in all tests (N=25) the muscle of the males sustained loads from 139.2 to 1531.2 g and in most tests (N=15) the muscles sustained 1670.4 g. The heaviest load sustained by any muscle was 1948.8 g.

The pattern of correlation between velocity of the muscle from the female and the loads sustained by the muscle (fig. 52) was similar to that of the male, as the load was increased the velocity decreased. The maximum velocity measured for the muscle of the female was 11.59±1.92 $mm.s^{-1}$, with a load of 203.6 g and the minimum velocity measured was 1.63±0.74 $mm.s^{-1}$, when the load was 1018.0 g (table 22). Extrapolation of the load-velocity curve showed a maximum velocity of about 18 $mm.s^{-1}$ (fig. 52). The velocity of the muscle from the female reaches to zero at a maximum load of 1500 g, estimated by extrapolation of the load-velocity curve. As table 22 shows, in all tests (N=25) the muscle of the females sustained loads from 203.6 g to 814.4 g and in most tests (N=20) the muscles sustained 1018.0 g. The heaviest load sustained by any muscles was 1425.2 g.

Table 21: Shows the mean and STD of velocity ($mm.s^{-1}$) of the muscle from the males, when the muscle load was increased. Each column, from C1 to C5 shows the mean and STD of velocity for five tests on an individual muscle. C6 shows the mean and STD of all tests (N=25) on the muscles. In all tests N=25 except at 1670.4 g, N=15; 1809.6 g, N=12; 1948.8 g, N=5.

WEIGHT (g)	C1	C2	C3	C4	C5	C6
139.2	15.77 ±3.22	20.75 ±2.25	12.13 ±4.59	18.50 ±2.68	19.68 ±2.96	17.37 ±3.46
278.4	13.54 ±3.31	17.65 ±2.50	10.07 ±4.48	15.45 ±3.19	16.50 ±2.47	14.64 ±2.97
417.6	11.98 ±3.91	15.80 ±2.14	9.31 ±2.96	13.93 ±3.09	14.18 ±1.45	13.04 ±2.49
556.8	10.55 ±4.32	13.73 ±2.11	8.79 ±2.77	12.26 ±2.35	12.56 ±1.69	11.58 ±1.93
696.0	9.35 ±4.55	11.76 ±1.68	8.33 ±2.26	10.81 ±1.92	10.84 ±1.41	10.22 ±1.36
835.2	8.18 ±4.29	9.83 ±2.37	7.62 ±3.70	9.40 ±2.93	9.26 ±2.66	8.86 ±0.92
974.4	7.34 ±3.02	9.13 ±1.65	6.99 ±2.97	8.15 ±2.42	8.19 ±3.04	7.96 ±0.83
1113.6	6.38 ±3.50	8.17 ±2.10	6.62 ±2.28	7.41 ±3.14	7.59 ±2.65	7.23 ±0.73
1252.8	5.49 ±3.28	7.23 ±1.72	6.21 ±2.26	6.13 ±2.98	6.56 ±2.55	6.32 ±0.64
1392.0	4.72 ±3.10	6.54 ±1.40	5.81 ±2.44	5.23 ±2.76	5.68 ±2.70	5.60 ±0.68
1531.2	4.15 ±2.77	5.64 ±2.74	5.45 ±2.21	4.52 ±2.20	4.83 ±2.09	4.92 ±0.62
1670.4	-	4.70 ±1.96	5.14 ±2.34	3.41 ±1.44	-	4.42 ±0.90
1809.6	-	3.97 ±1.96	4.55 ±2.09	2.83 ±0.75	-	3.78 ±0.88
1948.8	-	-	4.34 ±1.83	-	-	4.34 ±1.83

Table 22: Shows the mean and STD of velocity ($mm.s^{-1}$) of the muscle from the females, when the muscle load was increased. Each column, from C1–C5 shows the mean and STD of velocity for five tests on an individual muscle . C6 shows the mean and STD of all tests (N=25) on the muscles. In all tests N=25 except at 1018 g, N=20; 1221.6 g, N=13; 1425.2 g, N=2. The * shows that the animal responded just to one test.

WEIGHT (g)	C1	C2	C3	C4	C5	C6
203.6	9.92 ±2.47	13.06 ±2.07	10.99 ±2.89	9.87 ±3.04	14.12 ±1.21	11.59 ±1.92
407.2	6.02 ±2.09	9.89 ±1.07	7.39 ±2.76	6.68 ±2.29	7.75 ±2.66	7.55 ±1.47
610.8	3.73 ±0.89	6.65 ±2.00	5.16 ±1.85	4.35 ±1.97	4.25 ±1.26	4.83 ±1.14
814.4	2.63 ±0.97	4.10 ±0.70	3.47 ±1.49	2.38 ±1.66	2.38 ±1.15	2.99 ±0.77
1018.0	1.12 * –	2.86 ±0.40	1.80 ±0.42	1.05 ±0.48	1.34 ±0.43	1.63 ±0.74
1221.6	–	1.71 ±0.63	1.31 ±0.41	0.84 ±0.13	1.23 ±0.42	1.27 ±0.36
1425.2	–	–	0.65 ±0.39	–	–	0.65 ±0.39

The velocity of the male muscle was much higher and the muscle sustained much heavier loads than that of the female. The variation in velocity between individual muscles of the males was much greater than that of the females. Variation in velocity of both the males and females was greater when the muscles were loaded with lighter loads than with heavier ones (table 21, 22; fig. 51, 52).

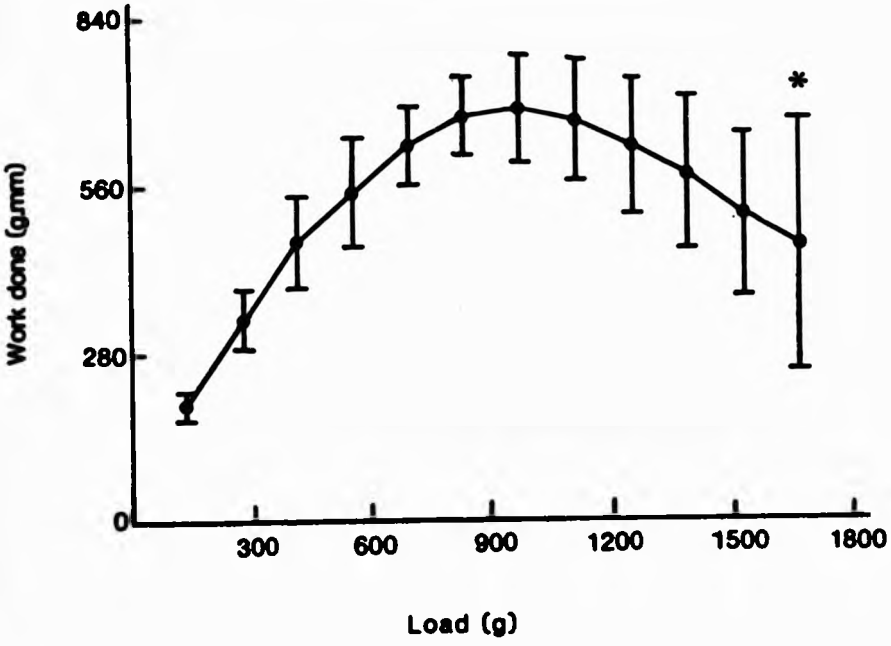
3.4.2. Work done during initial contraction

As the load was increased the work done by the muscle of the male during initial contraction increased, until it reached a peak. After that as the load increased the work done decreased (fig. 53). The peak work done during initial contraction was 690.0±89.0 g.mm, when the muscle sustained a load of 974.4 g (table, 23). The heaviest load that the muscle of most males sustained was 1670.4 g, where the work done was 464.4±211.4 g.mm. The heaviest load sustained by any muscles was 1948.8 g. The initial work done by the male reaches zero at a maximum muscle load of 2450 g, estimated by extrapolation of the load-work done. The measured minimum work done during initial contraction was 196.6±25.3 g.mm, when the muscle load was 139.2 g (table 23; fig. 53).

The correlation between load and the work done during initial contraction by the muscle of the female was similar to that of the male. However the work done by the female reached a peak much earlier than that of the male (fig. 53, 54). Peak work done during initial contraction by the females was

Fig. 53: Shows the correlation between work done (mean+/-STD) during initial contraction and increased load in isotonic contraction for the muscle of the males. As the load was increased the work done during initial contraction increased until it reached a peak, with further increased load the work done decreased. In all tests N=25 except for 1670.4 g (*) where N=15.

Fig. 54: Shows the correlation between work done (mean+/-STD) during initial contraction and increased load in isotonic contraction for the muscle of the females. As the load was increased the work done during initial contraction increased until it reached a peak, with further increased load the work done decreased. In all tests N=25 except for 1018 g (*) where N=20.


840
560
280
0
Work done (g.mm)
300
600
900
1200
1500
1800
Load (g)
*

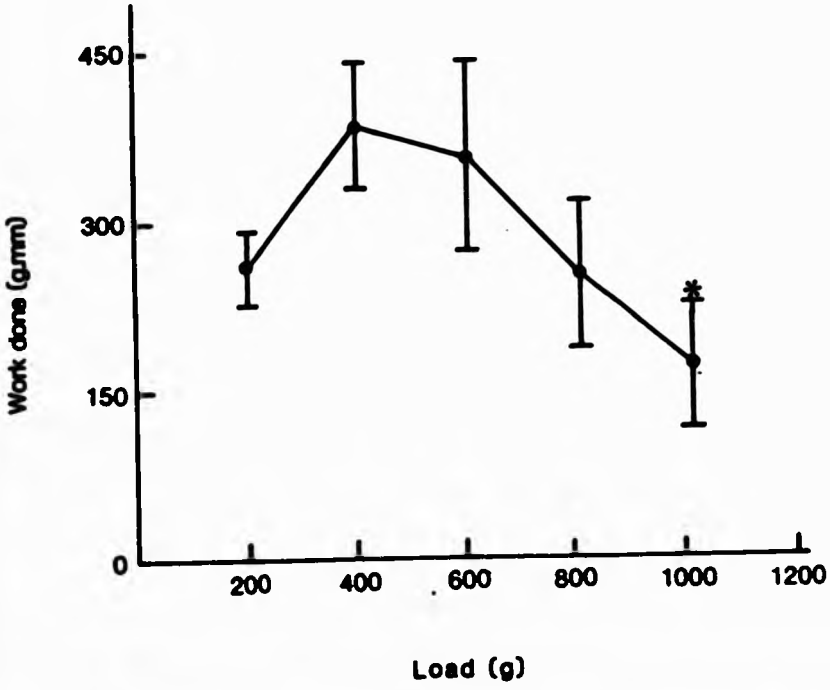

450
300
150
0
Work done (g.mm)
200
400
600
800
1000
1200
Load (g)
*

Table 23: Shows the mean and STD of work done (g.mm) during initial contraction by the muscle of the males, when the muscle load was increased. Each column from C1-C5 shows the mean and STD of the work done during initial contraction for five tests on an individual muscle. C6 shows the mean and STD of the work done during initial contraction in all tests (N=25) on the muscles. In all tests N=25 except at 1670.4 g, N=15; 1809.6 g, N=12; 1948.8 g, N=5.

WEIGHT (g)	C1	C2	C3	C4	C5	C6
139.2	196.0 ±48.8	210.2 ±32.7	157.6 ±72.3	193.5 ±50.2	225.6 ±27.8	196.6 ±25.3
278.4	362.4 ±118.9	358.3 ±62.5	255.4 ±110.4	361.6 ±90.0	372.9 ±98.0	342.1 ±48.8
417.6	485.4 ±189.3	481.3 ±94.7	342.7 ±137.5	486.6 ±117.9	554.6 ±133.0	470.1 ±77.5
556.8	547.4 ±256.0	589.1 ±130.9	418.6 ±261.7	534.2 ±163.4	671.5 ±183.6	552.2 ±91.9
696.0	603.8 ±335.6	663.2 ±147.3	558.8 ±332.6	600.2 ±209.9	727.2 ±246.9	630.6 ±65.6
835.2	620.4 ±367.4	718.6 ±203.9	649.4 ±498.6	629.3 ±252.8	776.2 ±367.6	678.8 ±66.7
974.4	605.6 ±369.6	754.0 ±194.9	759.6 ±391.0	580.4 ±166.4	750.4 ±312.1	690.0 ±89.0
1113.6	584.0 ±390.4	709.4 ±239.4	800.3 ±567.1	555.5 ±301.6	711.7 ±374.2	672.2 ±100.9
1252.8	529.9 ±393.6	657.0 ±285.9	795.2 ±482.6	514.4 ±302.5	650.9 ±389.0	629.5 ±113.8
1392.0	470.4 ±367.1	613.2 ±163.5	784.4 ±458.6	473.6 ±294.4	572.8 ±466.4	582.9 ±128.7
1531.2	438.2 ±322.0	561.4 ±334.4	732.6 ±512.1	425.9 ±289.2	409.6 ±243.2	513.6 ±136.4
1670.4	-	440.2 ±344.5	686.9 ±413.9	266.2 ±207.6	-	464.4 ±211.4
1809.6	-	439.4 ±299.0	602.7 ±306.0	248.3 ±101.1	-	430.1 ±177.4
1948.8	-	-	548.8 ±384.3	-	-	548.8 ±384.3

384.3±56.5 g.mm, when the load sustained by the muscle was 407.2 g. The heaviest load sustained by any muscles was 1425.2 g (table 24). The initial work done by the muscle of the female reaches zero at a maximum load of 1500 g, estimated by extrapolation of the load-work done. The minimum measured work done during initial contraction by the female was 170.0±55.5 g.mm, when the load sustained by most muscles was 1018.0 g (fig. 54, table, 24)

Peak work done during initial contraction by the male was much greater than that of the female as the male sustained much heavier loads. Whereas the work done during initial contraction by the female was relatively much greater when the muscles sustained lighter loads (table 23, 24; fig. 53, 54). As the standard deviation showed, the variation in the work done during initial contraction between the individual muscles from the females was greater than that of the males when the muscles sustained lighter loads (table 23, 24).

3.4.3. Work done during delayed contraction

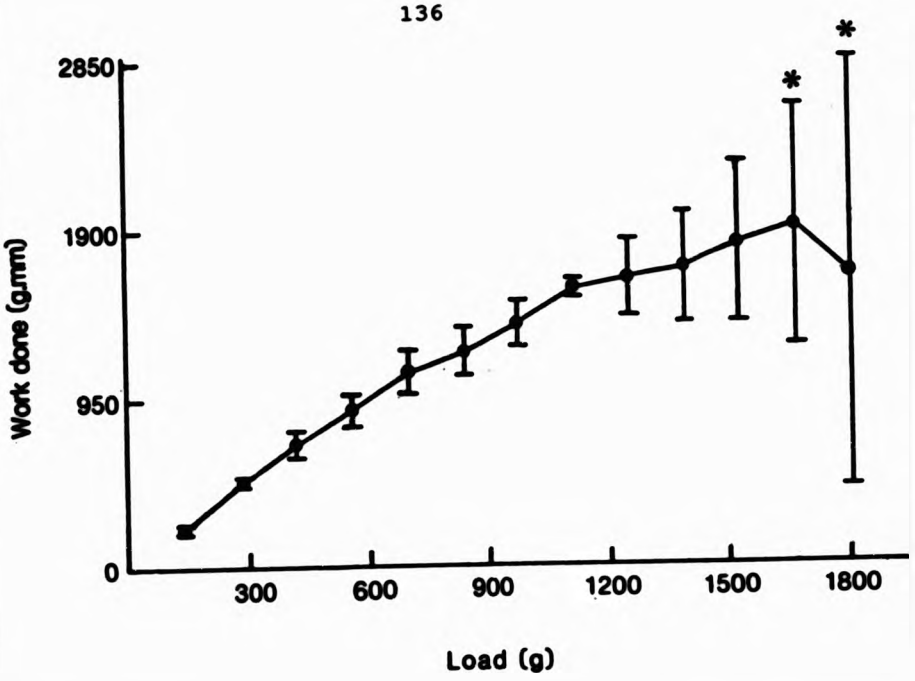
As the load was increased the work done during delayed contraction by the muscle of the male increased until it reached a peak, at 1670.4 g, which was close to the heaviest load that the muscle could sustain. With further increased muscle load the work done decreased (fig, 55). The measured mean peak work done during delayed contraction by the male was 1907.6±673.8 g.mm (table, 25). The heaviest load which most muscles sustained and at which the work done could be measured

Table 24: Shows the mean and STD of work done (g.mm) during initial contraction by the muscle of the females, when the muscle load was increased. Each column from C1-C5 shows the mean and STD of the work done during initial contraction for five tests on an individual muscle. C6 shows the mean and STD of the work done during initial contraction in all tests (N=25) on the muscles. In all tests N=25 except at 1018 g, N=20; 1221.6 g, N=13; 1425.2 g, N=2. The * shows that the animal responded only to one test.

WEIGHT (g)	C1	C2	C3	C4	C5	C6
203.6	249.2 ±103.6	272.4 ±26.8	287.1 ±127.1	206.5 ±91.4	280.6 ±127.2	259.2 ±32.7
407.2	299.4 ±184.9	360.9 ±56.7	446.4 ±239.5	401.4 ±219.1	413.7 ±202.4	384.3 ±56.5
610.8	219.0 ±94.7	334.8 ±68.5	398.5 ±248.8	403.7 ±163.1	427.9 ±192.3	356.8 ±84.4
814.4	148.5 ±42.2	297.3 ±95.5	281.9 ±57.3	300.3 ±236.5	229.1 ±91.4	251.4 ±64.3
1018.0	94.0 * -	248.4 ±115.3	162.8 ±52.0	185.2 ±172.8	159.5 ±49.5	170.0 ±55.5
1221.6	-	169.0 ±67.1	167.4 ±40.3	141.0 ±22.9	142.8 ±5.1	155.0 ±15.2
1425.2	-	-	151.2 ±27.7	-	-	151.2 ±27.7

Fig. 55: Shows the correlation between work done (mean+/-STD) during delayed contraction and increased load in isotonic contraction for the muscle of the males. As the load was increased the work done during delayed contraction increased until it reached a peak. With further increased load the work done decreased. In all tests N=25 except 1670.4 g, N=15; 1809.6 g, N=12, which have been shown by *.

Fig. 56: Shows the correlation between work done (mean+/-STD) during delayed contraction and increased load in isotonic contraction for the muscle of the females. As the load was increased the work done was increased until it reached a peak. With further increased load the work done was decreased. In all tests N=25 except for 1018 g (*) where N=20.


2850
1900
950
0
Work done (g/mm)
300
600
900
1200
1500
1800
Load (g)
*
*

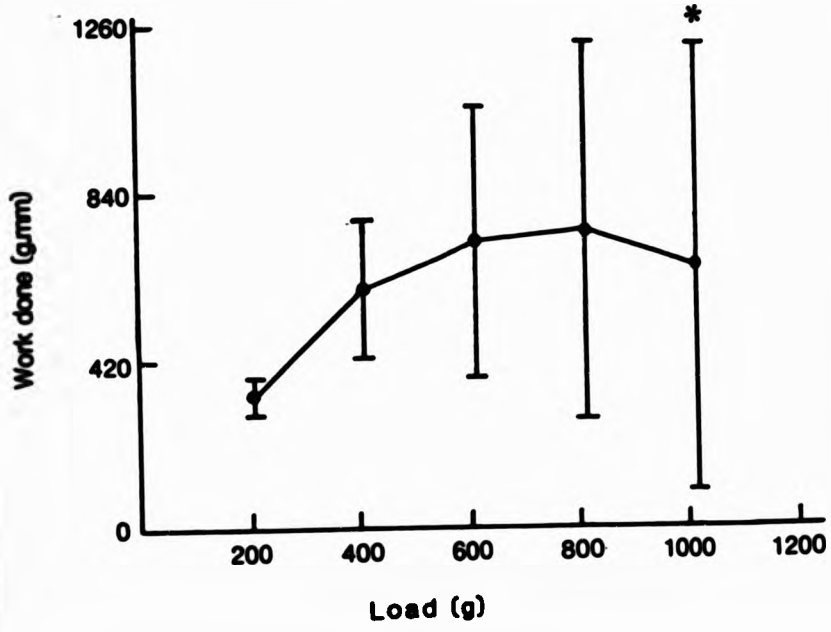

1260
840
420
0
Work done (g/mm)
200
400
600
800
1000
1200
Load (g)
*

Table 25: Shows the mean and STD of work done (g.mm) during delayed contraction by the muscle of the males, when the muscle load was increased. Each column from C1-C5 shows the mean and STD of the work done during delayed contraction for five tests on an individual muscle. C6 shows the mean and STD of the work done during delayed contraction in all tests (N=25) on the muscles. In all tests N=25 except at 1670.4 g, N=15; 1809.6 g, N=12, 1948.8 g N=5.

WEIGH (g)	C1	C2	C3	C4	C5	C6
139.2	259.1 ±18.2	214.0 ±65.9	210.1 ±31.6	253.4 ±25.0	229.4 ±88.7	233.2 ±22.3
278.4	514.8 ±63.0	457.4 ±222.9	469.7 ±82.6	505.3 ±65.7	476.2 ±155.1	484.7 ±24.4
417.6	760.8 ±96.5	678.4 ±336.5	580.2 ±175.1	701.2 ±146.8	761.5 ±131.6	696.4 ±74.6
556.8	958.4 ±184.9	881.4 ±442.5	731.4 ±384.7	918.9 ±337.0	929.8 ±202.2	884.0 ±89.7
696.0	1202.2 ±203.9	1093.4 ±541.2	895.0 ±430.0	1175.2 ±281.4	1122.6 ±286.4	1097.7 ±121.1
835.2	1386.0 ±280.8	1270.6 ±659.9	1086.0 ±663.7	1081.7 ±605.8	1225.0 ±380.7	1209.8 ±129.1
974.4	1466.9 ±390.9	1444.0 ±765.2	1416.0 ±597.4	1170.4 ±741.4	1331.4 ±695.8	1365.7 ±120.6
1113.6	1581.1 ±483.4	1632.6 ±891.7	1588.8 ±746.2	1510.7 ±789.1	1510.4 ±796.4	1564.7 ±53.2
1252.8	1602.0 ±856.5	1749.6 ±960.5	1835.3 ±745.0	1258.9 ±841.3	1651.0 ±874.5	1619.4 ±220.7
1392.0	1639.2 ±975.5	1872.8 ±1053.1	1952.0 ±954.1	1161.2 ±825.6	1753.2 ±844.3	1675.7 ±311.1
1531.2	2134.4 ±464.5	1974.7 ±1029.2	2302.1 ±491.9	1371.0 ±875.6	1300.6 ±941.1	1816.6 ±454.5
1670.4	-	2285.3 ±652.3	2307.8 ±610.9	1129.7 ±1045.9	-	1907.6 ±673.8
1809.6	-	2164.2 ±1173.4	2499.6 ±716.6	248.3 ±101.1	-	1637.4 ±1214.6
1948.8	-	-	2516.1 ±582.3	-	-	2516.1 ±582.3

was 1809.6 g, where the work done was 1637.4±1214.6 g.mm. In all tests (N=25) on the muscle of the males the muscles sustained loads from 139.2 g to 1531.2 g and in most tests (N=15) the muscles sustained 1670.4–1809.6 g. The heaviest load which any muscle sustained was 1948.8 g. The minimum work done measured during delayed contraction, was 233.2±22.3 g.mm, when the muscle load was 139.2 g (fig. 55; table, 25).

The work done during delayed contraction by the muscle from the female increased as the load was increased until it reached a peak, at 814.4 g, which was close to the heaviest load that the muscles could sustain (fig. 56). The peak work done by the muscles during delayed contraction was 739.9±469.8 g.mm (table, 26). The heaviest load which most muscles sustained was 1018.0 g in which the work done by the muscles, was 646.3±555.7 g.mm. In all tests (N=25) the muscles sustained loads from 203.6 g to 814.4 g and in most tests (N=20) the muscles sustained 1018.0 g. The heaviest load that any muscles sustained was 1425.2 g. The minimum work done measured for the muscle from the females during delayed contraction was 330.2±46.9 g.mm, when the muscle sustained 203.6 g (table, 26; fig. 56).

The peak work done by the muscle of the male during delayed contraction was much greater than that of the female, as the muscle from the male sustained much heavier loads. However the work done during delayed contraction by the muscle from the female was relatively greater than that of the male, when the

Table 26: shows the mean and STD of work done (g.mm) during delayed contraction by the muscle of the females, when the muscle load was increased. Each column from C1-C5 shows the mean and STD of the work done during delayed contraction for five tests on an individual muscle. C6 shows the mean and STD of the work done during delayed contraction in all tests (N=25) by the muscles. In all tests N=25 except 1018 g, N=20; 1221.6 g, N=13; 1425.2 g, N=4. The * shows that the animal responded only to one test.

WEIGHT (g)	C1	C2	C3	C4	C5	C6
203.6	318.0 ±108.8	319.1 ±28.6	350.3 ±38.2	395.5 ±44.7	268.2 ±83.3	330.2 ±46.9
407.2	477.0 ±283.5	432.9 ±33.8	658.0 ±197.9	863.9 ±59.9	553.8 ±241.7	597.1 ±171.9
610.8	334.3 ±260.5	369.2 ±71.8	945.1 ±277.0	1063.2 ±407.5	852.5 ±392.9	712.9 ±338.2
814.4	207.0 ±71.5	288.2 ±109.6	1044.3 ±511.4	1268.0 ±678.9	892.0 ±579.4	739.9 ±469.8
1018.0	94.0 * -	225.0 ±94.5	989.0 ±620.4	1428.8 ±791.6	494.5 ±497.1	646.3 ±555.7
1221.6	-	169.0 ±67.1	538.5 ±383.0	1291.2 ±767.1	307.8 ±228.3	576.6 ±500.2
1425.2	-	118.3 ±56.4	217.0 ±65.3	-	-	167.7 ±69.8

muscles sustained lighter loads. The work done by the muscle from the female and the male when they were loaded with lightest load were, 330.2±46.9 g.mm and 233.2±22.3 g.mm respectively. The variation in work done during delayed contraction by the muscle from the females was much greater than that of the males. As load was increased the variation of the work done by the muscles from both the male and female was usually increased (fig. 55, 56; table 25, 26).

3.4.4.The measured power output and work output

The maximum and minimum measured power output produced by the muscle of the male were 41.17±14.13 $W.Kg^{-1}$, when the muscle load was 556.8 g and 24.77±10.21 $W.Kg^{-1}$, when the load was 139.2 g respectively. The maximum and minimum power output produced by the muscle of the female were 52.01±26.85 $W.Kg^{-1}$, when the muscle load was 203.6 g and 6.50±6.39 $W.Kg^{-1}$, when the load was 1018.0 g respectively.

The maximum and minimum measured work output of the muscle from the male were 3.4±0.97 $J.Kg^{-1}$, when the muscle load was 556.8 g and 1.64±0.63 $J.Kg^{-1}$, when the load was 1670.4 g respectively. The maximum and minimum work output produced by the muscle of the female were 4.33±0.97 $J.Kg^{-1}$, when the muscle load was 203.6 g and 0.60±0.48 $J.Kg^{-1}$, when the load was 1018.0 g respectively.

3.4.5. Tension at maximum load

The calculated tension produced at maximum sustained load by

the muscles of the male and female were 4182 $g.g^{-1}$ and 5770 $g.g^{-1}$ respectively. The tension relative to the cross sectional area of the muscles of the male and the female were 1077 $g.cm^{-2}$ and 1336 $g.cm^{-2}$ respectively.

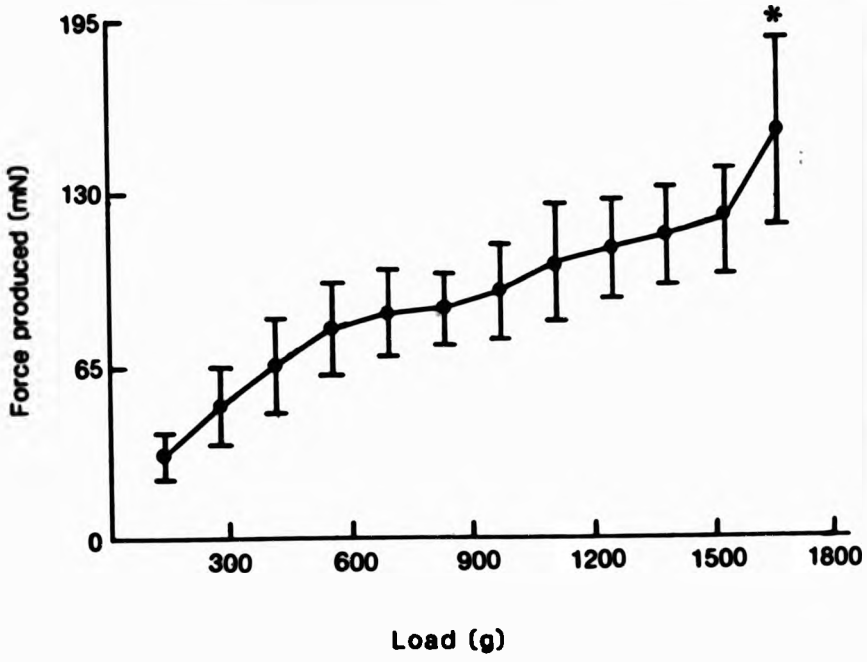
3.4.6. Force produced during initial contraction

As the muscle load of the male was increased the force produced increased until it reached a peak (fig. 57). Peak force produced by the muscle of the male was 152.4±35.1 mN, when the load was 1670.4 g which was the heaviest load sustained by most muscles (table 27). The minimum force measured for the muscle of the male was 31.2±8.6 mN, when the muscle load was 139.2 g. In all tests (N=25) the muscles sustained loads from 139.2 g to 1531.2 g and in most tests (N=15) the muscles sustained 1670.4 g. The heaviest load sustained by any muscles, was 1948.8 g (table, 27; fig. 57).

As the muscle load of the female was increased the force produced by the muscle increased until it reached a peak at 610.8 g, after that as the load increased the force produced fell (fig. 58). Peak force produced by the female was 30.6±13.6 mN. The heaviest load which most muscles of the females sustained was 1018.0 g in which the minimum force measured was 19.1±11.3 mN. In all tests (N=25) the muscles sustained loads from 203.6 to 814.4 g and in most tests (N=20) the muscles sustained 1018.0 g. The heaviest load sustained by any muscles was 1425.2 g (table, 28; fig. 58).

Fig. 57: Showing the correlation between force produced (mean+/-STD) during initial contraction and the muscle load, when the load was increased, in isotonic contraction for the muscle of the males. As the load was increased, the force produced by the muscle increased until the heaviest load that the muscle sustained. In all tests N=25 except for 1670.4 g (*) where N=15.

Fig. 58: Showing the correlation between force produced (mean+/-STD) during initial contraction and the muscle load, when the load was increased, in isotonic contraction for the muscle of the females. As the load was increased, the force produced by the muscle increased until it reached a peak. With further increases load the force produced decreased. In all tests N=25 except for 1018 g (*) where N=20.


195
130
65
0
Force produced (mN)
*
300
600
900
1200
1500
1800
Load (g)

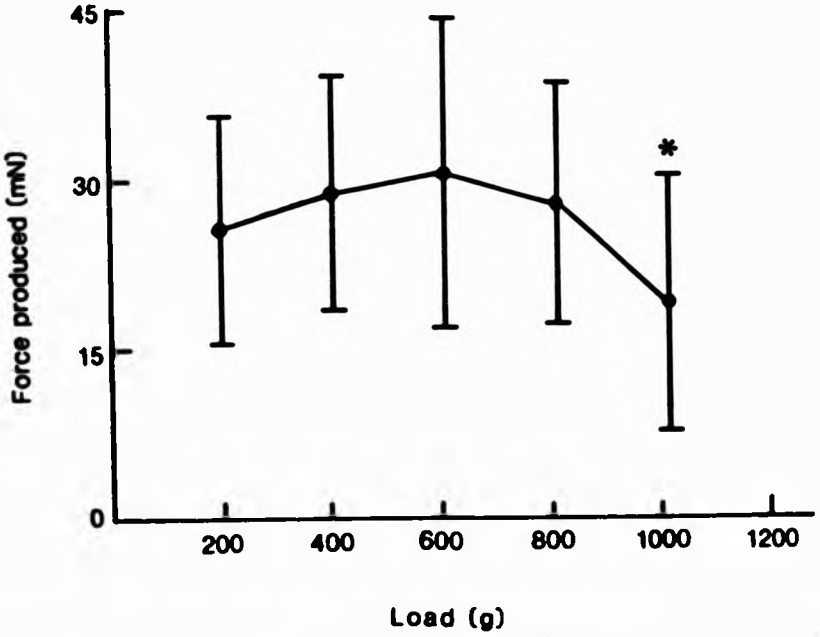

45
30
15
0
Force produced (mN)
*
200
400
600
800
1000
1200
Load (g)

Table 27: Showing the mean and STD of the force (mN) produced during initial contraction of the muscle from the males, when the muscle load was increased. Each column from C1–C5 shows the mean and STD of the force produced during initial contraction for five tests on an individual muscle. C6 shows the mean and STD of the force produced by the all tests (N=25) on the muscles. In all tests N=25 except at 1670.4 g, N=15; 1809.6 g, N=12; 1948.8 g, N=5.

WEIGHT (g)	C1	C2	C3	C4	C5	C6
139.2	24.81 ±4.76	40.21 ±7.21	19.71 ±9.04	37.24 ±16.47	33.97 ±9.38	31.19 ±8.63
278.4	39.52 ±6.90	68.19 ±12.97	31.57 ±16.62	54.42 ±21.01	57.44 ±7.34	50.23 ±14.62
417.6	52.05 ±13.25	91.09 ±11.20	46.59 ±19.51	72.13 ±25.02	64.56 ±7.13	65.28 ±17.59
556.8	64.53 ±26.46	101.61 ±23.24	62.42 ±19.73	90.89 ±26.87	74.64 ±9.47	78.82 ±17.00
696.0	71.69 ±29.77	103.16 ±22.74	67.50 ±14.21	99.49 ±29.15	82.10 ±12.13	84.79 ±16.06
835.2	77.65 ±36.82	99.59 ±36.96	69.49 ±30.81	99.34 ±25.65	84.31 ±33.22	86.08 ±13.30
974.4	88.98 ±22.92	107.88 ±26.76	65.65 ±31.03	109.88 ±37.63	91.16 ±39.68	92.71 ±17.84
1113.6	88.91 ±43.67	120.12 ±32.22	76.12 ±24.36	129.32 ±57.86	104.98 ±18.45	103.89 ±21.84
1252.8	94.93 ±49.87	133.28 ±28.42	86.99 ±51.99	119.17 ±62.06	109.76 ±19.41	108.83 ±18.55
1392.0	99.57 ±57.25	137.34 ±31.10	91.95 ±44.27	114.92 ±57.28	124.01 ±66.33	113.56 ±18.30
1531.2	95.11 ±60.19	137.15 ±62.31	104.63 ±34.13	119.04 ±33.14	139.57 ±46.15	119.10 ±19.56
1670.4	-	177.08 ±113.01	112.30 ±45.25	167.88 ±95.26	-	152.42 ±35.05
1809.6	-	128.66 ±51.84	113.88 ±52.28	106.48 ±12.16	-	116.34 ±11.29
1948.8	-	-	151.92 ±59.50	-	-	151.92 ±59.50

Table 28: Showing the mean and STD of the force (mN) produced during initial contraction of the muscle from the females, when the muscle load was increased. Each column from C1-C5 shows the mean and STD of the force produced during initial contraction for five tests on an individual muscle. C6 shows the mean and STD of the force produced by the all tests (N=25) on the muscles. In all tests N=25 except at 1018.0 g, N=20; 1221.6 g, N=13; 1425.2 g, N=2. The * shows that the animal responded only to one test.

WEIGHT (g)	C1	C2	C3	C4	C5	C6
203.6	18.60 ±6.69	26.19 ±6.64	19.85 ±7.94	20.86 ±7.92	42.75 ±36.97	25.65 ±9.99
407.2	28.11 ±24.57	46.15 ±11.54	21.98 ±7.09	20.10 ±7.45	27.55 ±12.81	28.78 ±10.31
610.8	28.72 ±21.02	53.51 ±29.26	29.74 ±15.75	22.92 ±15.90	18.05 ±10.75	30.59 ±13.60
814.4	32.82 ±15.57	41.32 ±18.33	30.74 ±19.65	16.46 ±14.39	17.96 ±13.18	27.86 ±10.51
1018.0	13.73 * -	37.41 ±8.41	21.72 ±8.92	8.68 ±7.11	13.83 ±9.87	19.07 ±11.26
1221.6	-	29.66 ±15.60	17.91 ±10.27	7.79 ±3.59	16.43 ±10.14	17.95 ±8.99
1425.2	-	-	6.20 ±5.76	-	-	6.20 ±5.76

The measured maximum force produced per gram by the muscle of the male and female were 327.1 $mN.g^{-1}$ and 123.9 $mN.g^{-1}$ respectively and the force produced relative to cross sectional area of the muscle of the male and female were 84.2 $mN.cm^{-2}$ and 28.6 $mN.cm^{-2}$ respectively. The muscle of the male produced much greater force than that of the female. The variation between the muscle of the males was usually greater than that of the females (fig. 57, 58; table 27, 28).

3.4.7. Angle at which the highest tension produced.

The muscle of some males produced peak tension at the flexion angles of 1.05 rad and that of others at 1.22 rad. The mean percentage peak tension, 59.83±3.33% produced by all tests (N=30), occurred at the angle of 1.22 rad (table 29), where the tibia was almost perpendicular to the longitudinal axis of the femur i.e. when the femur-tibia joint angle was close to the joint angle of the normal standing position on a flat surface. As the flexion angle of the femur-tibia was decreased from 2.45 rad, the tension produced increased, until the tension reached a peak, after that as the angle was decreased the tension also decreased (fig. 59).

Table 29: Showing percentage tension (mean±S.E.M) produced by the muscle at various flexion angles of the femur-tibia joint. Each column, from C1 to C6 shows, the percentage tension developed by the muscle of an individual animal (N=5). C7 shows percentage tension produced by the all tests (N=30).

Angle (rad)	C1	C2	C3	C4	C5	C6	C7
0.35	7.84 ±0.16	30.13 ±0.78	10.22 ±0.64	15.04 ±0.21	9.63 ±0.30	17.40 ±0.70	15.04 ±1.50
0.52	28.47 ±1.48	39.45 ±1.67	16.40 ±0.75	28.31 ±1.54	25.09 ±1.46	28.30 ±0.55	27.67 ±1.35
0.70	53.21 ±0.30	48.56 ±3.21	22.81 ±0.89	40.79 ±1.53	37.76 ±2.17	42.47 ±1.08	40.93 ±1.90
0.87	58.38 ±0.23	52.43 ±1.66	27.80 ±1.84	56.22 ±2.45	59.55 ±0.90	45.75 ±0.91	50.02 ±2.18
1.05	65.73 ±0.06	55.50 ±1.80	39.19 ±1.55	59.96 ±0.81	64.81 ±0.44	49.79 ±1.29	55.83 ±1.84
1.22	58.90 ±1.04	55.67 ±1.80	61.11 ±1.95	58.50 ±1.19	59.21 ±0.99	65.61 ±0.11	59.83 ±0.61
1.40	47.74 ±0.80	49.30 ±1.65	50.08 ±1.21	55.31 ±1.37	53.59 ±1.12	61.13 ±0.57	52.86 ±0.90
1.57	44.96 ±0.63	45.62 ±1.44	47.96 ±2.34	50.60 ±0.86	49.42 ±1.20	54.57 ±0.46	48.85 ±0.64
1.75	30.14 ±1.05	43.31 ±2.10	34.82 ±1.26	49.16 ±0.80	44.66 ±0.89	42.41 ±0.80	40.75 ±1.27
1.92	23.05 ±0.50	35.63 ±1.93	25.21 ±1.20	42.89 ±0.73	33.58 ±1.18	38.53 ±1.35	33.15 ±1.40
2.10	18.07 ±1.05	31.36 ±1.25	22.59 ±0.70	39.56 ±2.50	28.76 ±0.49	33.59 ±1.19	29.05 ±1.43
2.27	15.90 ±0.67	28.42 ±0.82	21.96 ±0.74	35.66 ±1.05	23.77 ±0.62	26.49 ±0.85	25.37 ±1.21
2.45	15.15 ±0.44	26.06 ±0.64	18.07 ±0.89	14.43 ±0.41	19.66 ±0.51	22.45 ±0.16	19.30 ±0.81

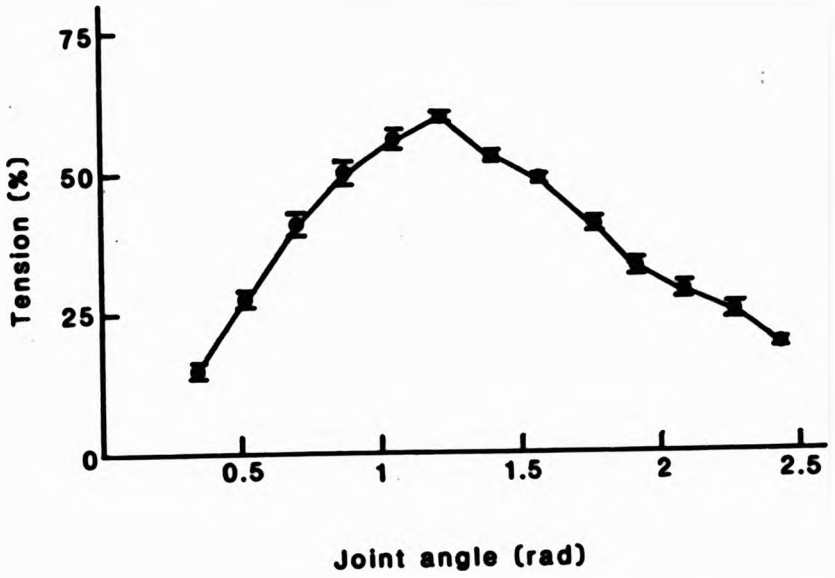


Fig. 59: showing the pattern of correlation between the flexion angle of the femur-tibia joint and the tension produced (mean± S.E.M, N=30), by the muscle. It also shows the flexion angle at which the muscle produces the peak tension, N = 30.

3.5. MECHANICAL PROPERTIES OF THE FEMUR-TIBIA ARTICULATION

The velocity of muscle contraction during tibial flexion from a femur-tibia joint angle of 1.57 rad to full flexion was determined, from traces of tibial movement with time displayed on an oscilloscope. The distance of tibial movement was corrected for linearity by the calibration of the transducer. The mean and STD of the distance which the tibia moved during flexion at steps of 5 msec was measured, from five traces of tibial movement at three different loads (Fig. 60). Since both the transducer and the muscle were connected to a common lever, the tibia, muscle movement is proportional to the transducer displacement. Therefore the muscle shortening during the flexion was determined by dividing the tibial movement by the lever factor of 6.96. From these data the velocity of muscle shortening was examined by plotting shortening distance against time for three different loads (Fig. 61).

The duration of total muscle shortening from a femur-tibia joint angle of 1.57 rad to total flexion of the tibia was, approximately 165 msec, 200 msec and 170 msec when the muscle sustained loads of 139.2 g, 974.4 g and 1809.6 g respectively. The maximum muscle shortening was, 2.63±0.21 mm, 1.33±0.09 and 0.23±0.025 mm, when the loads were 139.2 g, 974.4 g and 1809.6 g respectively.

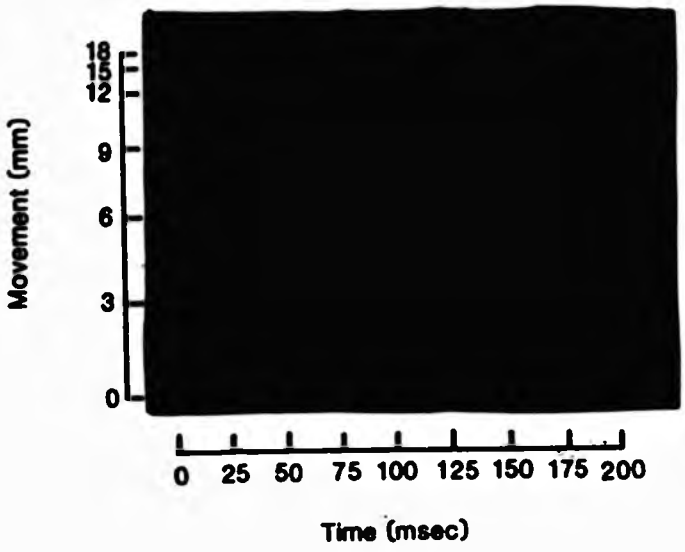
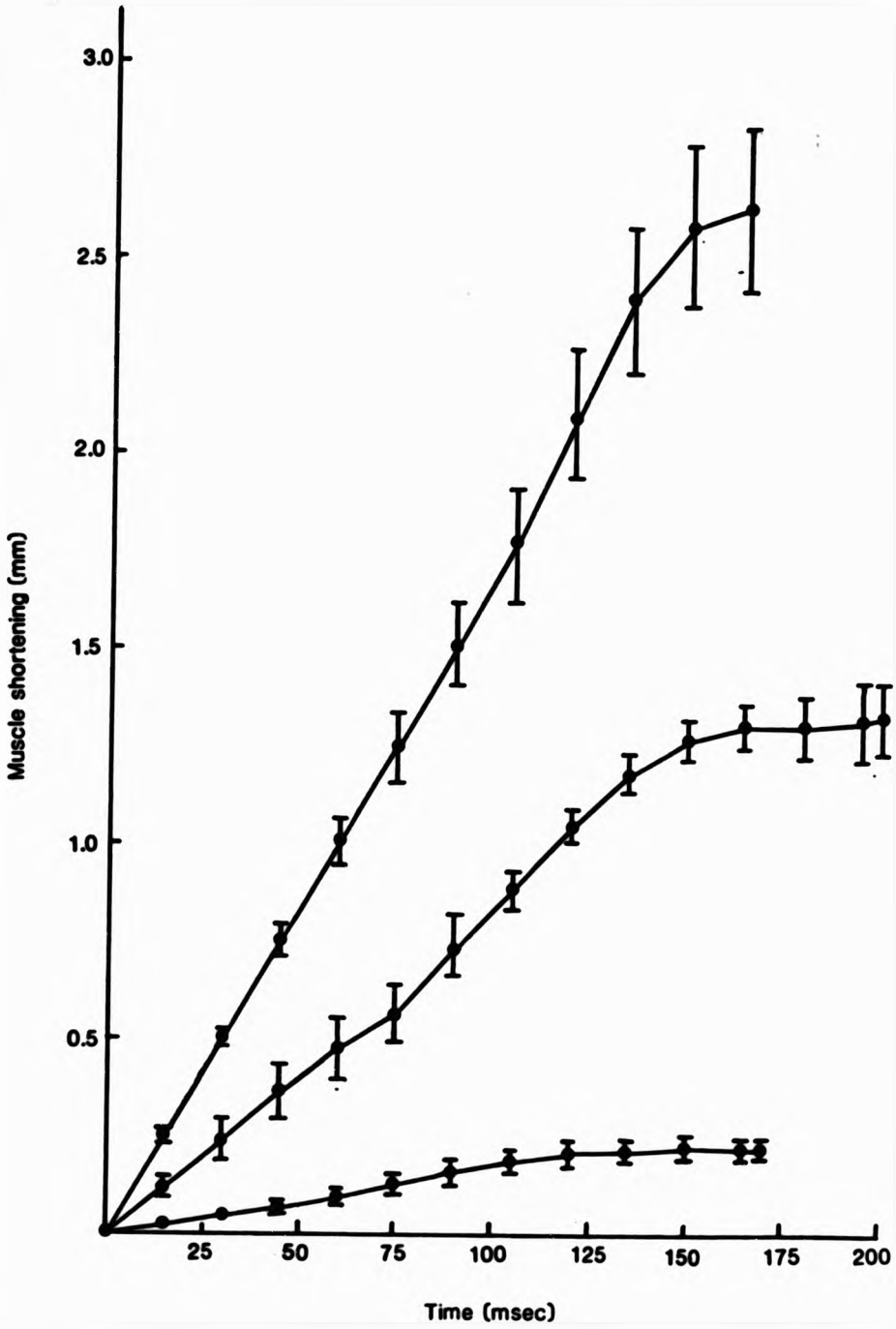


Fig. 60: An example of the traces on the screen of the an oscilloscope showing tibial movement with reference to time at a specified load, five traces are shown for the same loading, from a femur-tibia joint angle of 1.57 rad to full flexion of the tibia, when the muscle load was 139.2 g. The horizontal scale shows the duration of movement, each section is 25 msec. The vertical scale shows the distance of tibial movement, each section is 3 mm (note that this scale is not linear).

Fig. 61 shows the velocity of muscle shortening during tibial flexion, from the femur-tibia join angle of 1.57 rad to full flexion of the tibia, when the muscle sustained load of 139.2 (upper curve), 974.4 g (medial curve) and 1809.6 g (lower curve).


3.0
2.5
2.0
1.5
1.0
0.5
Muscle shortening (mm)
25
50
75
100
125
150
175
200
Time (msec)

The comparison of the muscle velocity throughout the contraction, under different loads showed that muscle velocity was more constant at lower loads (fig. 61). The results showed that muscle velocity under a load of 139.2 g, was almost constant throughout the contraction. Therefore the velocity of contraction in the unloaded muscle, during normal behaviour, would be quite constant throughout the contraction.

The mechanical properties of the male metathoracic femur-tibia joint during the tibial flexion were examined through steps of 0.35 rad. The mechanical properties were also determined with respect to time and muscle moment arm which were related to flexion angle. The angle of maximum extension of the tibia, 2.45 rad, was taken as the angle at which the strike started. The parameters were assumed to be zero at the start of the strike i.e. at maximum extension of the tibia. Although the linearity of the muscle contraction was determined from a femur-tibia joint angle of 1.57 rad to full contraction, however, it was assumed in all calculations that the flexor tibialis muscle is contracting at the maximum initial velocity of 22.5 $mm.s^{-1}$ (see section 3. 4. 1), throughout the flexion from full extension, 2.45 rad, to full flexion tibia. The mean and standard error of mean (S.E.M) of each subject were calculated from 10 measurements of the moment arm.

3.5.1. The Moment arm

As the flexion angle was decreased, from 2.45 rad, the moment

arm increased from 1.8±0.09 mm until it reached a peak of 2.46±0.09 mm at flexion angle of 1.05 rad. After that as the angle was decreased the moment arm also decreased until it reached 1.89±0.06 mm, when the tibia was fully flexed (table, 30; fig. 62).

3.5.2. The mechanical properties of the leg joint and flexor tibialis muscle during tibial flexion

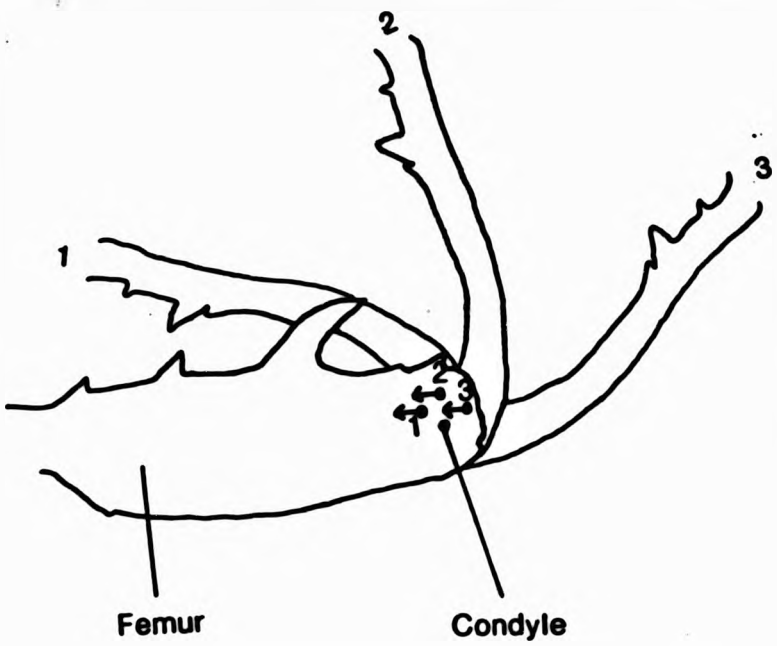
As the joint angle decreases from full extension, the angular velocity of the tibia will initially show little change but from 1.0 rad it will increase rapidly (Fig. 63). As figure three shows, the distance of muscle shortening required to produce equal angular displacements of the tibia is smaller at full extension and more particularly at full flexion. The relationship between the muscle shortening and angular movement of the tibia is not linear. The minimum calculated angular velocity of 9.35±0.43 rad.s^{-1} will occur at a flexion angle of 1.4 rad. The maximum velocity of 175±5.55 rad.s^{-1}, will occur when the tibia approaches the end of the strike, at an angle close to full flexion of the tibia (table, 30; fig. 63). The velocity during initial closing of the femur-tibia joint will be almost constant, with the exception of the first step of movement, from 2.45 rad to 2.1 rad at which the velocity will rise relatively quickly to 18.75±1.01 rad.s^{-1} and then fall (fig. 63).

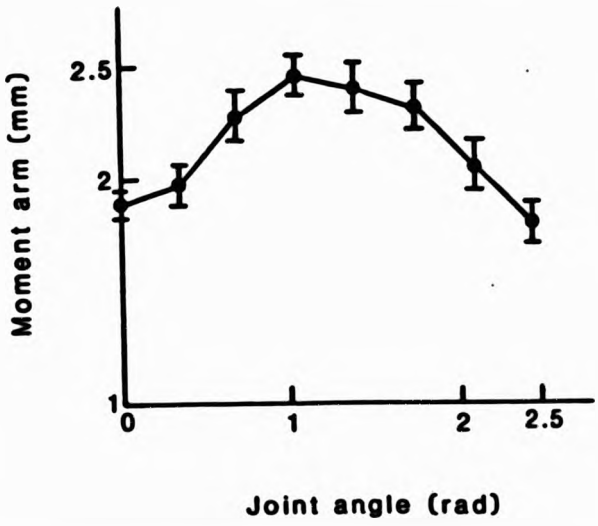
The angular acceleration of the tibia will follow the pattern described above during flexion but peaks before full flexion

Table 30: Shows the moment arm of the femur-tibia joint throughout 0.35 rad steps of the tibial flexion and the calculated time at maximum velocity for each angular movement of the tibia. The table also shows the calculated angular velocity and acceleration of the tibia at each flexion angle throughout the strike (mean±S.E.M, N=10).

Joint angle (rad)	Moment arm (mm)	Time of flexion (ms)	Angular velocity ($rad.s^{-1}$)	Angular acceleration ($rad.s^{-2}$)
2.45	1.80 ±0.09	0	0	0
2.10	2.06 ±0.11	14.0 ±0.75	18.75 ±1.01	526.5 ±28.4
1.75	2.32 ±0.10	42.0 ±1.81	10.74 ±0.46	−157.5 ±6.7
1.40	2.40 ±0.11	81.0 ±3.71	9.35 ±0.43	24.5 ±1.1
1.05	2.46 ±0.09	117.0 ±4.28	12.15 ±0.44	1153.0 ±41.8
0.70	2.28 ±0.11	141.0 ±6.8	65.62 ±3.16	14483.0 ±697.4
0.35	1.97 ±0.09	144.0 ±6.58	145.83 ±6.66	20661.0 ±943.6
0	1.89 ±0.06	146.0 ±4.63	175.00 ±5.55	14585.0 ±462.6

Fig. 62: Shows the relation between the active point attachment of the flexor tibialis muscle to the proximal part of the tibia (1, 2 and 3) and the condyle, during the flexion tibia. The distances from number 1, 2 and 3 to the condyle are the moment arms; when the tibia was totally flexed, when the femur-tibia joint angle was 1.57 rad and when the tibia was totally extended respectively (upper diagram). The correlation between the flexion angle of the femur-tibia joint and the moment arm (mean±S.E.M, N=10). As the joint is flexed the moment arm increases until it reaches a peak, after that the moment arm decreases with further decrease in the angle (lower diagram).


2
3
1
Femur
Condyle


Moment arm (mm)
2.5
2
1
0
1
2
2.5
Joint angle (rad)

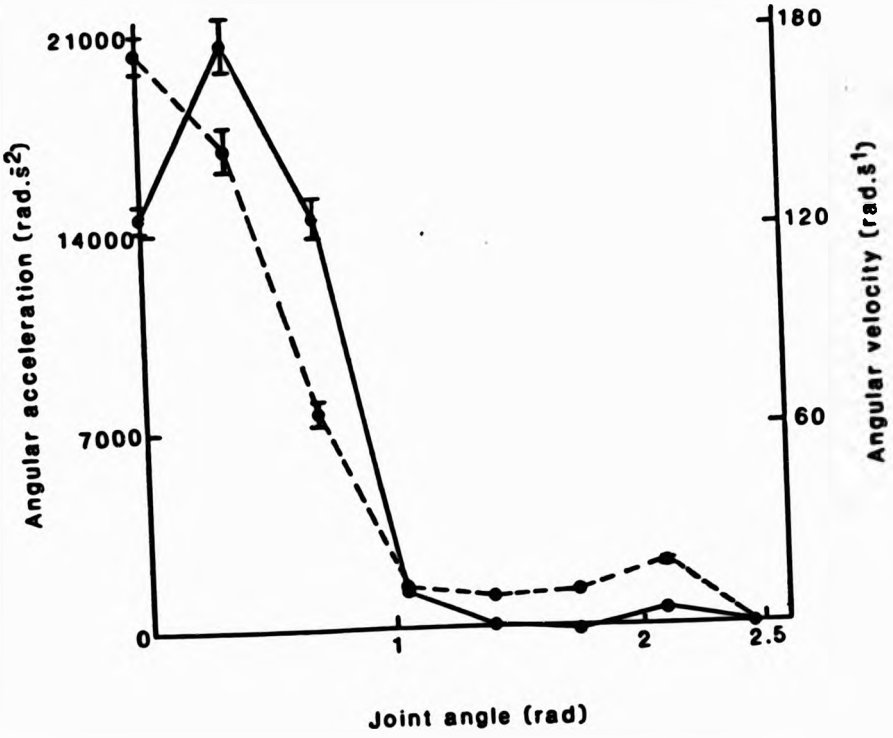


Fig. 63: Shows the correlation between the flexion angle of the femur-tibia joint and both the angular velocity (dashed line) and the angular acceleration (solid line) of the tibia (mean±S.E.M, N=10), during the defensive strike. As the flexion angle is decreased the velocity and acceleration increase exponentially throughout the strike.

(Fig. 63). There will be almost no acceleration during initial closing of the femur-tibia joint, with the exception of the first step from 2.45 rad to 2.1 rad where the acceleration will rise relatively quickly, to 526.5±28.4 $rad.s^{-2}$, and then fall to an estimated -157.5±6.7 $rad.s^{-2}$, at the angle of 1.75 rad. At the flexion angles below 1 rad the acceleration will rise quickly and below 0.35 rad fall until the tibia reaches total flexion. The maximum calculated acceleration of 20661.0±943.6 $rad.s^{-2}$ occurs at the flexion angle of 0.35 rad (table, 30; fig. 63). This indicates that flexion occurs in two phases; 1-Constant velocity at initial closing of the femur-tibia joint. 2-Rapidly increasing velocity at later closing of the joint. The maximum calculated acceleration occurs within 144±6.58 msec of start of the movement (table, 30). It is estimated that the flexion of the tibia will be completed in 146±4.63 msec.

During flexion, the kinetic energy of the tibia will increase proportional to the cube of angular velocity. Kinetic energy (E_k) in erg is given by equation 1 from Burrows (1969).

1- $$E_k = 1/2\ I.w^2$$

Where w is the angular velocity in $rad.s^{-1}$ and I is the moment of inertia in $g.degree^{-1}$ which is given by equation 2

2- $$I = 1/3\ m.L^2$$

Where m is mass of the tibia in gram and L is length of the tibia in cm. Therefore the kinetic energy of the tibia will

increase through the defensive strike as does the angular velocity.

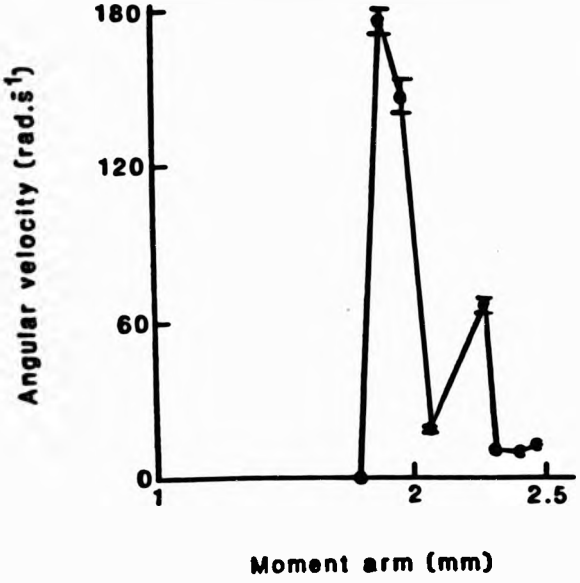
The angular velocity and acceleration of the tibia vary considerably with joint angle and therefore with time during flexion.

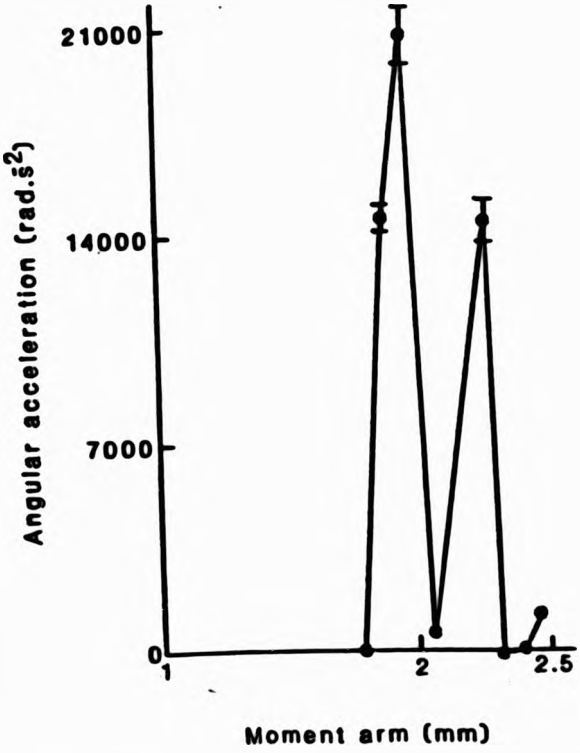
The predicted relationship between angular velocity of the tibia and muscle moment arm is complex (Fig. 64; table 30). The velocity will initially rise to a calculated peak of 175.0 $rad.s^{-1}$, at a moment arm of 1.89 mm, then falls until it reaches 18.75 $rad.s^{-1}$, at a moment arm of 2.06 mm. On further increases in the moment arm the velocity will rise to a second peak of 65.62 $rad.s^{-1}$ at a moment arm of 2.28 mm. The velocity will generally be lower at longer moment arms i.e. the moment arms of the femur-tibia joint angles at which the muscle produced higher tension (see section, 3.4.7). The minimum angular velocity of the tibia will occur at the moment arm of 2.40 mm and the maximum velocity at moment arm of 1.89 mm (table, 30). The correlation between the muscle moment arm and the kinetic energy of tibia movement during flexion will be similar to the correlation between the moment arm and angular velocity, because the energy changes proportional to the cube of velocity.

The calculated change in angular acceleration with muscle moment arm exhibits a similar bimodal peak (fig. 65; table, 30). The acceleration will rise to an estimated initial peak

Fig. 64: Shows the correlation of the moment arm of the muscle with angular velocity of the tibia (mean±S.E.M, N=10). The first ascension in the graph, from strike initiation, has been drawn by extrapolation.

Fig. 65: Shows the correlation of the moment arm of the muscle with angular acceleration of the tibia (mean±S.E.M, N=10). The first ascension in the graph, from strike initiation, has been drawn by extrapolation.


180
120
60
0
1
2
2.5
Angular velocity (rad.s-1)
Moment arm (mm)


21000
14000
7000
0
1
2
2.5
Angular acceleration (rad.s-2)
Moment arm (mm)

of 20661±943.6 rad.s^{-2}, at a moment arm of 1.97 mm, then fall to 526.5±28.4 rad.s^{-2} at a moment arm of 2.06 mm. After that it will rise to a second peak of 14483±697.4 rad.s^{-2} at moment arm of 2.28 mm, then the tibia will decelerate to -157.5±6.7 rad.s^{-2} at a moment arm of 2.32 mm and again the acceleration will rise. The acceleration of the tibia related to the moment arm will have two phases due to the acceleration being lower at shorter moment arms (Fig, 65).

The correlation between the torque acting about the femur-tibia articulation, applied by the flexor muscle in moving the tibia, and the angular acceleration is given by equation 3 (Burrows, 1969),

3- $$\Gamma = I.\alpha$$

where Γ is the torque in dyne.cm.rad^{-1}, α is the angular acceleration of the tibia in rad.s^{-2} and I is moment of inertia in g.$degree^{-1}$. The moment of inertia, I (equation 2) is constant throughout tibial flexion, this has a value of 0.47 g.$degree^{-1}$, since the mass, 0.19 g, and length, 2.72 cm, of the tibia are constant. Therefore the relationship between the torque and femur-tibia joint angle will be similar to the relationship between the angular acceleration and the joint angle. The torque applied by the muscle to give the maximum tibial acceleration, 20661 rad.s^{-2}, will be Γ=20661x0.47 =9711 dyne.cm.rad^{-1}.

The distance between femur and tibial spines was measured at each flexion angle of the tibia. The femur-tibia joint angle

from full extension, 2.45 rad, to 1.8 rad was too great to hold an object between the spines. The largest estimated joint angle at which the insect can hold a cylindrical object between the femur and tibial spines was estimated 1.8 rad, at which the distance between femur and tibial spines was 13 mm. If the caught object has diameter smaller than 13 mm it will fall in the femur-tibia joint and hold between the spines. Whereas the object with diameter greater than 13 mm, can not be held between the femur and tibial spines and the object will come out between the spines. The insect can catch and hold the head, beak or neck of small bird, chick, or the lip of some mammals, monkey. However the insect may catch and hold a soft object with slightly larger diameter than 13 mm, by driving the main tibia and femoral spines into the restrained object.

The angular velocity calculated for various flexion angles show that, E. calcarata can catch an object of 13 mm in diameter, with an angular velocity of about 12 $rad.s^{-1}$ and angular acceleration about zero, at flexion angle of 1.8 rad. Whereas an object of 4 mm in diameter would be caught with a velocity of about 20 $rad.s^{-1}$ and angular acceleration about 3000 $rad.s^{-2}$, at flexion angle of 1 rad (fig. 63).

The relationship between force produced by the muscle and the femur-tibia joint angle during tibial flexion will be similar to the pattern between the angular acceleration of the tibia and the joint angle. The correlation between acceleration and

force generated by a movement is given by equation 4

4- $$F=m.a$$

where F is the force produced by the movement to give the acceleration, m is the mass of the object which moves and a is the acceleration of the movement. As mentioned above the acceleration of the tibia during flexion at lower flexion angle will be higher, hence force required by the muscle to give the higher tibial acceleration at lower flexion angles will be higher.

4. DISCUSSION

4.1. Histochemical properties of the muscle

Histochemical tests are good indicators of muscle fibres diversity and distribution within muscles. Muscle fibres are commonly classified into 3 wide categories of fast, intermediate and slow based on their structural, contractile, electrical and biochemical properties (Atwood, 1976). The correlation between histochemical, ultrastructural and functional capacities of the muscle fibres of crustaceans and insects has been demonstrated by some authors (Jahromi and Atwood, 1967, 1969b; Kent and Govind, 1981; Morgan and Stokes, 1978; Morgan et al, 1980; Ogonowski and Lang, 1979; Stokes, 1987; Stokes et al, 1979).

Muscle fibres which stain lightly for nicotine adenine dinucleotide-tetrazolium reductase, NADH-TRase, activity and darkly for myosin adenosine triphosphatase, ATPase, activity are considered to be fast, indicating that these fibres have a low oxidative capacity which is related to the low density of mitochondria, however they exhibit a high specific activity of the myosin ATPase which is related to the high density of myosin filaments. The fibres that stain lightly for ATPase activity and darkly for NADH-TRase activity are considered to be slow (Govind et al, 1981; Kent and Govind, 1981; Morgan et al, 1980; Ogonowski and Lang, 1979; Ogonowski et al, 1980; Stokes, 1987; Stokes et al, 1979). Stokes, et al (1979) have

classified fibres within the mesocoxal muscles of the cockroach, Periplaneta americana; some fibres which stain with intermediate density for ATPase and oxidative enzymes, are fast contracting and fatigue resistant, the fibres which develop the most positive reaction with oxidative enzymes have a slow contraction and are fatigue resistant, those which developed the least reaction with oxidative enzymes were found to be fast contracting and fast fatiguing. Stokes, et al (1979) described another type of fibres which stain with low density for myosin ATPase and oxidative enzymes, they proposed that these fibres would exhibit slow contraction but fatigue rapidly.

The histochemical tests, for ATPase and NADH-TRase activity, indicate that the muscles studied here are composed of both fibres with darker and lighter staining. The majority of fibres stained darker for ATPase and lighter for NADH-TRase activity, indicating fibres with fast contraction and fast fatigue. Some fibres stained lighter for ATPase and darker for NADH-TRase activity, particularly in regions DMA and DMP, indicating fibres with slow contraction and fatigue resistance. Moreover there were some fibres which stained darker for both ATPase and NADH-TRase activities especially in some dorsal regions, suggesting these are fibres with fast contraction and fatigue resistance. Therefore on histochemical evidence the muscles studied here are a mixture of fibres with fast contraction and fast fatigue (approximately 50%), fibres with fast contraction and fatigue resistance (approximately

35%) and fibres with slow contraction and fatigue resistance (approximately 15%).

A number of authors have studied the distribution of fast and slow fibres within crustacean and insect muscles (Govind et al, 1981, 1986b; Kent and Govind, 1981; Morgan and Stokes, 1978, 1979; Ogonowski et al, 1980; Stokes et al, 1979). They show that some crustacean and insect muscles are homogeneous, containing slow or fast fibres and others are heterogeneous, containing both slow and fast fibres. In heterogeneous muscles slow or fast fibres are located in particular regions of the muscles. The muscles examined here for ATPase activity showed that most fibres stained darker i.e. they are faster fibres. The small groups of fibres that stained lighter were scattered throughout the muscles, however they were more frequent in the dorsal regions. When the muscles were examined for NADH-TRase activity, most fibres stained lighter i.e. they are faster fibres. The small groups of fibres which stained darker were scattered throughout the muscle but more frequent in dorsal regions. I am not aware of any study on crustacean or insect muscle which shows that slow and fast fibres are distributed widely throughout the muscle as is seen in E. calcarata.

The muscle fibres tested here for NADH-TRase activity exhibited a uniform staining pattern, which correlates with the distribution of mitochondria throughout the cross section of the fibres, seen in ultrastructural studies, between myofibrils. The pattern contrasts with that of crustacean

muscles e.g. with that of the closer muscle of the first walking leg of the lobster Homarus americanus (Govind et al, 1981), with that of the closer muscles in the major and minor claws in the fiddler crabs (Govind et al, 1986b) and also contrasts to the staining pattern of superficial, slow, flexor and extensor abdominal muscles fibres of the lobster and crayfish Procambarus (Ogonowski and Lang, 1979). In these muscles slow fibres stain principally at the periphery, around the sarcolemma. This pattern being determined by the high density of subsarcolemmal mitochondria. The staining pattern for NADH-TRase of the muscle fibres studied here, however is similar to those of other insects e.g. to that of the mesocoxal muscles of cockroach Periplaneta americana, shown by Stokes et al (1979). The staining pattern for the ATPase activity in E. calcarata is uniform throughout the cross sectional area, which correlates with the distribution of myosin filaments. The pattern is similar to previous findings.

The density of staining for ATPase and NADH-TRase activities may also correlate with fibre diameter. Slower fibres which stain with lower density for ATPase activity have smaller diameters than faster fibres that stain darker for ATPase activity (Ogonowski and Lang, 1979; Prosser, 1973; Stokes, 1987). The muscles examined here for ATPase and NADH-TRase activity also showed that larger fibres stain darker for ATPase activity and lighter for NADH-TRase activity.

This study suggests that the flexor tibialis muscles of both

the male and female E. calcarata are a mixture of physiological fibre types with predominantly fast fibres and the muscle of the male contains more fast fibres than that of the female.

4.2. Ultrastructure of the muscle

In this study the physiology of individual muscle fibres was not studied but the knowledge of specific relationships between ultrastructural and physiological properties of muscle fibres allows us to predict physiological fibre types within the muscle from the ultrastructural results.

The Analysis of Variance (ANOVA) is a statistical technique which is used to determining whether more than two means differ significantly from one another, or in other words, whether more than two samples are drawn from the same population. Whereas the T-test can be used to compare the means of two samples. Therefore, the ANOVA was performed, for all ultrastructural parameters, to determine the degree of homogeneity between the 12 sample parts within the muscles of the male and the female and between the whole muscle of the male and the female. Since the histochemical results showed that the fibres which stained darkly for ATPase and lightly for NADH-TRase were more frequent in dorsal region than in the ventral one, the ANOVA test was also performed to determine whether the populations in the dorsal and ventral regions differ significantly from one another. The ANOVA was followed by T-test to determine the degree of significance between different muscle parts.

The sliding-filament hypothesis of muscle contraction which was first proposed by Huxley and Niedergerke (1954), predicts

that muscle fibres with longer sarcomere will produce higher maximum tension per cross sectional area and are slower than fibres with short sarcomere length, with the assumption that the myofilaments of two fibres have equivalent number of similar cross-bridges per unit length. If the force between thick and thin filament is generated at each of a series points in the region of overlap in each sarcomere, the tension per filament will be proportional to the number of these points i.e. to the width of overlapping region, which is related to sarcomere length. Fibres with shorter sarcomeres contract faster than those with longer sarcomeres, due to the larger number of sarcomeres in series within a given length of muscle fibres.

The mean sarcomere lengths of the muscle of E. calcarata, 5.44 µm for the male and 5.27 µm for the female, are similar to those of crustacean and insects. The mean sarcomere lengths of the prothoracic flexor tibialis muscle of the stick insect Carausius morosus, and the metathoracic extensor tibialis of the locust Locusta migratoria migratorioides, measured by Huddart and Oates (1970) is about 4.5 µm. The mean sarcomere lengths of the femoral extensor muscle from cockroach Leucophaea maderae fabricius, measured by Hagopian (1966), is 5.3 µm. The short, medium and long sarcomeres (mean sarcomere lengths in the muscle parts) within the muscle of E. calcarata, have similar lengths to short, 4 µm; medium, 5 µm; and long, 6-8 µm; sarcomeres within the carpopodite flexor muscle of the walking leg of the crab Portunus respectively

(Franzini-Armstrong, 1970).

Many authors have found that, the contractile properties of different fibres are strongly correlated with their sarcomere lengths (Fahrenbach, 1967; Franzini-Armstrong, 1970; Govind et al, 1981, 1986b; Hoyle, 1967, 1969; Jahromi and Atwood, 1967, 1969a,b; Pringle, 1972). Pringle (1972) stated that, in all observations sarcomere lengths in the slow muscles are longer than those within fast muscles. The sarcomere lengths of some parts within the muscles of both the male and the female E. calcarata are significantly different from others ($p < 0.001$), therefore both the muscles have heterogeneous population of muscle fibres. Most parts within the muscles of both the male and the female are significantly different from each other ($p=0.05$). The mean sarcomere lengths of fibres within the muscle parts of E. calcarata, with shortest, medium and longest sarcomeres are 3.97 µm, 5.11-5.29 µm and 6.3 µm for the male and 4.5 µm, 5.1 µm and 5.7 µm for the female. The mean sarcomere lengths in the muscle parts with short sarcomeres, are similar to sarcomere lengths of the phasic fibres from the accessory flexor muscle in the meropodite of the walking leg of Cancer magister, with length of 4.5 µm. The mean sarcomere lengths within the parts with long sarcomere, however, is much shorter than sarcomere lengths of tonic fibres of the muscle from the crab, with length of 12 µm, measured by Fahrenbach (1967). The results for E. calcarata, suggest that the muscle contains a range of physiological fibres types with minority of slow fibres. The fast and slow

fibres of the cutter closer muscle of Homarus americanus, have sarcomere lengths of <4 µm and >6 µm (Govind, 1982). On this basis the muscle of E. calcarata, appears to be composed a the majority of intermediately fast fibres, as most parts of the muscle have mean sarcomere lengths of between 4.5-6 µm.

Although the mean sarcomere lengths within dorsal region is shorter than the mean within the ventral regions, and in some regions of the flexor tibialis muscles there are more fibres with shorter sarcomeres than in other regions, however, the fibres with shorter or longer sarcomeres are not concentrated in particular areas. Most parts within both the dorsal and ventral regions are significantly different from each other. The range and STD within each individual parts also show that in same parts the different sarcomere lengths exist. This is different from extensor tibialis muscle fibres of the cockroach Periplaneta americana (Jahromi and Atwood, 1969b), in which the proximal region has longer sarcomeres, with a mean of 5.83 µm, and the central region has uniform and shorter sarcomeres, with a mean length of 4.75 µm. The location of fibres with long and short sarcomere within the muscle of E. calcarata, also contrasts with what Govind (1982) observed in the claw cutter closer muscle of the lobster, in which the fibres with long sarcomeres, slow fibres, are restricted to a small ventral region of the muscle. Pringle in (1972) reported that many crustacean muscles contain fibres with different sarcomere lengths and are mixed randomly. The

distribution of fibres with long and short sarcomere found in E. calcarata is similar to crustacean limb muscles, in which the muscles contain fibres with different sarcomere lengths and are mixed randomly.

The sarcomere lengths of the muscle of the male are significantly different to those of the female. The greater range and STD of sarcomere lengths within the muscles of the male and that the population of the dorsal region is significantly different from the ventral region in the male with lower level of probability than the female suggest that the muscle of the female is less heterogenous.

Several authors found that slow fibres have an irregular, wavy and thick Z line, however fast fibres have relatively regular, straight and thin Z lines (Cochrane et al, 1972; Franzini-Armstrong, 1970; Hagopian and Spiro, 1967; Huddart and Oates, 1970; Prosser, 1973). The Z lines in the flexor muscles of E. calcarata are wavy, whereas the Z lines in some fibres are thicker, more wavy and irregular than others.

There are several studies which show that larger fibres are faster than smaller fibres, however, I am not aware of any study which explains why! It may be because oxygen does not diffuse to central parts of large fibres sufficiently quickly and the oxidative metabolism of mitochondria located in the central parts of the fibres can not proceed at an adequate rate.

In order to make comparisons between the cross sectional area of muscle fibres measured here and those of other insects and crustaceans, I have converted the diameter of muscle fibres of the crustaceans and insects, commonly used by other authors, to the cross sectional area of the fibres, by assuming that all fibres are cylindrical.

Skeletal muscle fibres of crustaceans and insects exhibit a wide range of cross sectional areas (Elder, 1975; Hagopian, 1966; Hoyle, 1967, 1978; Pringle, 1972; Prosser, 1973). The range of cross sectional area of fibres within the muscle of the male E. calcarata, is from 582 μm^2 to 25,550 μm^2 and that of the female is from 2,407 μm^2 to 15,027 μm^2. Pringle (1972) reported that, the fibre diameter in arthropod ranges from 40 μm (1,256 μm^2 ca.) to 200 μm (31,400 μm^2 ca.). Whereas Elder (1975) stated that, only the diameter of insect limb muscle fibres range from 10 μm (79 μm^2 ca.) or less to 1 mm (0.79 mm^2 ca.) or more. The diameter of the extensor muscle, jumping muscle, fibres of the locust Schistocerca gregaria Forskal ranges from 40 μm (1,256 μm^2 ca.) to 140 μm (15,386 μm^2 ca.) (Hoyle, 1978). Therefore the range of cross sectional area of the muscle fibres of E. calcarata is within the range of arthropods and other insects. However the range of the muscle of the male is much greater than that of the locust, the muscle contains relatively larger fibres than the locust. The cross sectional area of fibres within the muscle of the male are significantly different from those within the female ($p < 0.001$), the male has larger mean fibres cross sectional

area than the female. The range and STD within the muscle of the male is also much greater than that of the female, the male has some much larger fibres than the female.

Correlation between the cross sectional area of muscle fibres and their function in crustacean and insects has been observed by several authors. Muscle fibres with larger cross sectional area are considered to be faster than those with smaller cross sectional area (Atwood, 1965; Elder, 1975; Hoyle, 1967, 1978; Huddart, 1975; Jahromi and Atwood, 1971; Pringle, 1972; Prosser, 1973; Reger and Cooper, 1967; Stokes, 1987; Usherwood, 1967). The histochemical tests on the muscles studied here also suggests that, the larger muscle fibres are faster as they stain darker for ATPase which is related to higher density of myosin filament and lighter for NADH-TRase which is related to lower density of mitochondria.

The muscles studied here contain fibres with a wide range of cross sectional areas, mentioned above. The cross sectional areas of the muscle fibres are much smaller than those of fast fibres of the main and distal superficial layers of the extensor muscle of the carpopodite in the walking leg of lobster Homarus americanus, which have diameter of 300-600 μm (70,650-282,600 μm^2 ca.), and are smaller than slow-followers of the lobster which have diameter of only 100-300 μm (7,850-70,650 μm^2 ca.) (Jahromi and Atwood, 1971). The cross sectional areas of the flexor muscle fibres are also much smaller than those of fast and intermediate fibres in the

closer muscle of the walking leg from the spider crab Chionectes tanneri, with diameter of 300-400 μm (70,650-125,600 μm^2 ca.) and 150-250 μm (17,663-49,063 μm^2 ca.) respectively. Whereas the areas are larger than those of slow fibres of the crab, with diameter of 50-130 μm (1,963-13,267 μm^2 ca.) (Atwood, 1965). similar to fast basalar fight muscle and much larger than slow extensor tibialis muscle fibres of Lepidopteran achalarus lyciades which have diameter of 100-150 μm (7,850-17,663 μm^2 ca.) and 50-100 μm (1,963-7,850 μm^2 ca.) respectively (Reger and Cooper, 1967). This suggests that muscle of E. calcarata has much slower fibres than the muscle of walking leg of the lobster and the crab but has much faster fibres than the extensor tibialis muscle of Lepidopteran achalarus lyciades. The fibres cross sectional area of some parts within the muscles of both the male and female E. calcarata are significantly different from others ($p < 0.001$), the muscles are heterogeneous. Most parts within the muscles of both the male and the female are significantly different from each other ($p=0.05$). The range and STD of cross sectional areas within the flexor tibialis are high. These predict that, the muscle contains different physiological fibre types.

The distribution of muscle fibres with different diameters vary within different muscles (Dorai Raj, 1964; Hoyle, 1978; Huddart, 1975; Morgan and Stokes, 1979; Theophilidis and burns, 1983). The range and STD within same muscle parts studied here showed that in same parts the fibres with

different cross sectional areas exist, the fibres with larger and smaller cross sectional area are scattered throughout the muscles. This differs from what have been observed in other insect muscles, in which the fibres with larger and smaller cross sectional area are grouped in separate regions of the muscles, the fibres with large and small cross sectional area are not mixed randomly e.g. the distribution in E. calcarata is different to that of the mesothoracic flexor muscle of locust (Theophilidis and Burns, 1983) and differs from coxal branch of the main depressor muscle (135d') of the cockroach (Morgan and Stokes, 1979). The greater range and STD within the muscle of the male and that the population in the dorsal region of the muscle of the male is significantly different from that of the ventral parts with lower level of probability than those of the female, support the suggestion that the muscle of the female has more uniform fibres distribution and population, with more slower fibres.

Hoyle (1978) suggests that, the difference between the fibre diameters within the extensor (jumping) muscle of the locust Schistocerca gregaria Forskal. is related to their locations on the metathoracic femur rather than fibre types. He observed that, the diameter decreases from proximal to distal parts with the larger being at the thickest part of the femur. It is unlikely that the thickness of different muscle fibres studied here are due to thickness of different regions of the femur, since the femur of E. calcarata is similar in thickness throughout.

Atwood (1965) observed in the muscle of the crab, the fast fibres have diameter of 300–400 μm (70,650–125,600 μm^2 ca.) and sarcomere length of 4.7±0.4 μm, however slow ones have a diameter of 50–130 μm (1,963–13,267 μm^2 ca.) and sarcomere length of 10.4±0.6. The correlation between sarcomere length and cross sectional area of the muscle fibres studied here is also seen. There are some parts of the muscle from the male which have a shorter sarcomere and larger cross sectional area e.g. DDP and also some parts which have a longer sarcomere and smaller cross sectional area e.g. VMP.

The thin to thick filament ratio and muscle fibres speed are correlated. The reason why the fibres with lower ratios are faster, may be related to the fact that myosin filaments in the active state make cross-bridge connections with the actin filaments. With low ratios, the smaller number of thin filaments surrounding each thick filament, may facilitate bridge formation between all, or most, thin filaments and a thick filament. In slow fibres with higher ratios the thin filaments may interfere to each other and decrease the number of bridge formed. In highly structured muscle fibres with more thick filaments and less thin filaments, thin filaments may not interfere with each other. The myosin ATPase activity is related to density of myosin filaments i.e. the faster rate of ATPase activity occurs with closer myosin filaments.

The mean ratio of the muscles studied here, 4.85:1 for the male and 4.92:1 for the female, and number of thin filaments

surrounding each thick filament, 9-12, are very similar to those found by several authors in insect and crustacean muscles (Elder, 1975; Franzini-Armstrong, 1970; Hagopian, 1966; Hagopian and Spiro, 1967; Pringle, 1972; Prosser, 1973; Smith et al 1966). The mean ratio of thin to thick filaments of fibres within the muscle parts studied here, are within the range of ratios for arthropod muscles. As Pringle (1972) reported that, the thin to thick filament ratio in arthropod muscles is always greater than 2:1 and the highest ratio is about 7:1. Smith et al (1966) described that, insect skeletal muscles have an orbit of 12 thin filaments surrounded each thick one and ratio of 5:1 or 6:1.

A number of authors have found, a correlation between the ratio of thin to thick filaments and arrangement of the filaments and physiological fibre types. The thin to thick filament ratio in fast fibres is lower than that of slow fibres (Fahrenbach, 1963, 1967; Govind et al, 1986a; Hoyle, 1967; Jahromi and Atwood, 1967, 1969a,b; Smith et al, 1966; Stokes, 1987). There are highly significant differences between some parts and others ($p < 0.001$) within the muscles of both the male and the female, the muscles are heterogeneous. Most parts in the muscles are significantly different from each other ($p=0.05$). The range of filament ratios, 2.77:1-8.68:1 for the male and 2.86:1-7.86:1 for the female, is great. The tonic fibres of extensor tibialis muscle in the metathoracic leg from the locust Schistocerca gregaria. has ratio of higher than 5:1, the phasic fibres of the

extensor and retractor unguis muscles in the locust have same ratio of 5:1 (Cochrane et al, 1972). Franzini-Armstrong (1970) reported ratios of 3.6:1 to 4.4:1 in the short sarcomere muscle fibres, and ratios of 4.8:1 and 5.5:1 in the long ones, in the carpopodite flexor muscle of the crab Portunus depurator. The parts with higher mean ratio and those with lower mean ratio have very similar ratio to those of insect and crustacean. Hence the most parts of the muscle from E. calcarata, have mean ratio from 4.7:1 to 5.65:1, similar to the higher ratio of the crustacean. This may suggest that the muscle fibres are relatively slower than those of crustaceans. The muscle contains predominantly slower fibres. The significant differences between different parts and great range of ratios within the muscles of both the male and the female may indicate that the muscles are a mixture of physiological fibre types. The population within the muscle of the male is not significantly different ($p=0.465$) from that within the female. The dorsal region population is significantly different from that of the ventral region within the muscles of both the male and the female, but with higher F ratio and lower level of probability for the male. This may suggest that the muscle of the female is less heterogeneous, the distribution and population of fibres within the muscle of the female is more uniform, with more slower fibres.

The thick filament number per μm^2 of fast fibres is greater than that of slow fibres. The thin and thick filaments number within both the muscles of the male and female showed that the

muscles are not homogeneous and that there are highly significant differences between some parts and others ($p < 0.001$). Most parts within the muscle are significantly different from each other ($p=0.05$). The range of thick filament number within the muscles, 250–1,025 per μm^2 for the male and 400–1,050 per μm^2 for the female, and also the range of thin filament number of fibres, 1,775–5,200 per μm^2 for the male and 2,250–5,375 per μm^2 for the female, are great. The muscles contain fibres with significantly different number of filaments. Jahromi and Atwood (1969a) found in deep (fast) and superficial (slow) abdominal muscle fibres of the lobster that, the fast fibres have 450 thick filaments per μm^2, however slow fibres have 350 thick filaments. On this basis the muscle of E. calcarata has much faster fibres than the abdominal muscles of the lobster, the lowest and highest mean thick filament number of fibres within the parts of the muscles studied here, are 471.2 and 848.8 per μm^2. The most fibres within the flexor muscles have also much higher thick filament number than fast fibres within the coxal muscles of the cockroach, in which fast fibres from coxal muscle (137) have 600 thick filaments per μm^2 and from muscle (135c) have 650 thick filaments per μm^2 (Jahromi and Atwood, 1969b). Hoyle (1967) observed that the most remarkable difference between the fast and slow fibres of the extensor tibialis muscle of Periplaneta americana is the difference between their thin filament number. Stokes et al (1975) also found that in dorsal longitudinal muscle of the katydid slow fibres have more thin filament than fast ones. The muscles studied here are a

mixture of fibres with significantly different filament ratios and filaments numbers per μm^2, indicating that the flexor tibialis muscles of E. calcarata contain a range of fibres from fast to slow. The thick filaments number within the muscle fibres indicates that the muscles contain relatively much faster fibres than other insects and crustaceans.

The thick filaments number per 100 μm^2 showed that the population in the dorsal region of the muscle of the male is not significantly different from that of the ventral region ($p=0.105$), however the dorsal region population within the female is significantly different from that of the ventral region ($p=0.001$). This may suggests that the male is less heterogeneous and that the distribution of fibre throughout the muscle is more uniform than the female. The thin filaments number shows that the population in the dorsal region within the male is significantly different from that of the ventral region ($p < 0.001$), but the population in the dorsal region of the female is not significantly different from that of the ventral region ($p=0.644$). This may suggest that the muscle of the female is less heterogeneous and the distribution and population of fibre types throughout the muscle is more uniform than the male, the female has more slower fibres.

The arrangement of thick and thin filaments and the number of thin filaments surrounding each thick filament in arthropod muscles, have been observed by several authors (Atwood, 1972; Elder, 1975; Fahrenbach, 1967; Hagopian, 1966; Hagopian and

Spiro, 1967; Hoyle and McNeill, 1968; Huddart and Oates, 1970; Jahromi and Atwood, 1969b; Smith et al 1966). In the muscles studied here, the thick and thin filaments arrangement and the number of thin filament surrounding each thick filament, are not same in all fibres. The thick filaments are arranged in a square array, however in some fibres are arranged more regularly, in perfect square array, than others. A circle of 9-12 thin filaments surround each thick filament. The previous observations show that the phasic fibres have a regular arrangement of filaments, in which each thick filament is surrounded by 6 thin filaments, ratio of 3:1. Whereas the tonic fibres have irregular arrangement of the filaments and each thick filament is surrounded by 9-12 thin filaments, ratio of 5:1 or 6:1 e.g. In the phasic fibres of the accessory flexor muscle of the walking leg of the crab Cancer magister, 6 thin filaments surround each thick one and the thick filaments are aligned in a hexagonal array. Whereas tonic fibres have an irregular arrangement of filaments, in which each thick filament is enveloped by 10-12 thin ones and in this case it is unlikely that thick filaments are in hexagonal array (Fahrenbach, 1967). Jahromi and Atwood (1967) found similar results in phasic and tonic fibres from abdominal extensor muscles of the crayfish, Procambarus clarki and Orconectes virilis and in the fast and slow abdominal muscle fibres of the lobster. On basis of the thin filament number surrounded each thick filament, the majority of the fibres within the muscle of the E. calcarata are similar to slow fibres of the crab, the lobster and the crayfish, in which

each thick filament is surrounded by 9–12 thin filament.

Most of the these authors describe or illustrate a myofilaments arrangement, in arthropod muscles, in which one thick filament is surrounded by more than 6 thin filaments, commonly 9–12 e.g. in the metathoracic extensor muscle of the locust, Locusta migratoria migratorioides (Huddart and Oates, 1970), in the prothoracic flexor tibialis muscle of the stick insect, Carausius morosus (Huddart and Oates, 1970), in the cockroach femoral muscles (Hagopian and Spiro, 1967) and in most cockroach coxal muscles (Usherwood, 1962). In arthropod muscles, other than flight muscles, the hexagonal arrangement, in which each thick filament is encircled by 6 thin filaments is not common. When the ratio is high, thick filaments are often not arranged in a regular hexagonal array. Whereas in fibres with a ratio of 6:1 i.e. when thick filaments surrounded by a circle of 12 thin filaments, thick filaments in a regular hexagonal array have been observed. The fastest of the short sarcomere muscle fibres of crustaceans have a regular arrangement of thick and thin filaments and the arrangement is similar to that of insect flight muscles (Atwood, 1972). The muscle of E. calcarata have both fibres with regular and irregular arrangement of filaments. This can also predict that the muscle is a mixture of fibre types. The number of thin filaments surrounding each thick filament are very similar to those of other insect muscles.

One of ultrastructural criteria examined here to predict the

physiological muscle fibre types was mitochondrial density. Mitochondria are necessary for oxidative metabolism in muscle fibres. Highly active, oxidative, muscles have high density of mitochondria e.g. mitochondria occupy up to 40% of the volume of slow muscle fibres (Elder, 1975), whereas there is much less mitochondria in glycolytic muscle fibres.

The amount of mitochondria and fibre types are strongly correlated (Ashhurst, 1967; Atwood, 1972; Elder, 1975; Hoyle and McNeill, 1968; Levenbook and Williams, 1956; Morgan and Stokes, 1978; Prosser, 1973; Smit et al, 1967; Usherwood, 1967; Vogell et al, 1959). The these authors demonstrated that fast fibres which fatigue rapidly contain a small amount of mitochondria, whereas slow fibres which have prolonged contraction contain large amount of mitochondria. The area and number of mitochondria of some parts within muscles of both the male and the female are highly significantly different from other ($p < 0.001$), the muscles are heterogeneous. Most parts within the muscles are significantly different from each other ($p=0.05$). The range of mitochondrial area of the whole muscles studied here, 0.79%-19.15% for the male and 3.27%-19.74% for the female, also the range of mitochondrial number per 100 μm^2 of the cross sectional area of the fibres, 7.66-68.91 for the male and 15.31-80.39 for the female, are great.

In the fast muscle 136 and ventral fibres of the 135d of the mesocoxal muscles of the cockroach Periplaneta americana,

mitochondria occupied 2.7% and 4.4% of fibres volume respectively, whereas the slow dorsal fibres of muscle 135d have 25.5% mitochondria (Morgan and Stokes, 1978). The muscle parts of E. calcarata with the largest mitochondrial area have only a mean area of 11.73% for the male and 14.78% for the female. Thus the muscles studied here primarily contain less aerobic fibres than the 135d of the cockroach. Usherwood (1967) compared red and white fibres of the retractor unguis muscle from the metathoracic leg of the locust Schistocerca gregaria. He found that both fibre types have similar mechanical and electrical properties but the white fibres fatigue more rapidly than the red ones. Elder (1975) stated that these red fibres have a volume of mitochondria double that of the white ones. Some authors have found high percentage volume (30-40%) of flight fibres occupied by mitochondria, which is much more than that found in leg muscles (Ashhurst, 1967; Elder, 1975; Jahromi and Atwood, 1969b; Smit et al, 1967; Smith, 1966; Vogell et al, 1959). The flight muscles are continuously active for long times, much longer than other muscles. Vogell et al (1959) compared flight muscle with jumping, extensor tibialis, muscle of the locust Locusta migratoria. They found that the flight muscles are rich in mitochondria, have very high aerobic metabolism and are active for a long time, however fast jumping muscle has brief activity and has few mitochondria, 5-10%. The mean percentage mitochondrial area of the whole muscles studied here are similar to that of the locust jumping muscle and much lower than flight muscles. The flexor tibialis muscle of the

male and the female are composed of fibres with significantly different mitochondrial area and mitochondrial number. Therefore the muscles are a mixture of physiological fibre types, with predominantly less aerobic fibres.

The number and percentage areas of mitochondria within the male is highly significantly different from the number and areas within the female ($p < 0.001$). The muscle of the female contains a larger amount of mitochondria, which suggests that the male has less aerobic fibres than the female. The areas and number of mitochondria of some dorsal parts in the male and the female are significantly different from some ventral parts ($p < 0.001$). The dorsal region in the male has larger area and higher number of mitochondria than ventral region, this may suggest that dorsal region of the male has more aerobic fibres than ventral one, however the ventral parts of the female has the larger area and higher number of mitochondria than dorsal parts, the ventral parts of the female has more aerobic fibres.

There are several studies on mitochondrial size, shape and location in muscle fibres. In crustacean and insect muscle fibres, mitochondria vary in their shape, size and locations (Atwood, 1972; Cochrane et al, 1972; Elder, 1975; Hagopian, 1966; Hagopian and Spiro, 1967; Huddart and Oates, 1970; Jahromi and Atwood, 1971; Reger and Cooper, 1967; Smith, 1962). The size of mitochondria of some parts are significantly different from others ($p < 0.001$) within muscles

of both the male and the female, the muscles are heterogeneous. Most parts are significantly different from each other ($p=0.05$). The range of mitochondrial size within the muscles studied here is great, 0.06 μm^2–0.51 μm^2 for the male and 0.11 μm^2–0.43 μm^2 for the female. The mitochondria are located between myofibrils and also are seen near the Z line. The mitochondria vary in shape being, oval, oblong or irregular. Hagopian (1966) found three types of mitochondria in the metathoracic extensor and flexor tibialis muscles of the cockroach, Leucophaea maderae fabricius, these are, the oval mitochondria that are located under the sarcolemma and are approximately 2 µm long and 0.6 µm in diameter, the elongated mitochondria that are situated between myofibrils, vary in length from 5 to 25 µm and are roughly of 0.5 µm diameter. A third type of mitochondria lies very close to the Z line and this type has three processes that appear in the interfibrillar sarcoplasm. Smith (1962) found large mitochondria of 5 µm in length, in the coxal muscles of Periplaneta, randomly distributed among the myofibril. Elder (1975) stated that, in insect muscles mitochondria are vary in their alignment being longitudinally arranged or randomly located among the myofibrils or arranged in pairs close to Z lines. In insect flight muscles mitochondria are located between myofibrils and are irregular in shape. Cochrane et al (1972) showed that, in the metathoracic extensor tibialis muscle of the locust Schistocerca gregaria, pairs of mitochondria are located in interfibrillar spaces close to Z line.

Some of the these authors described a correlation between the mitochondrial size and fibre types. Elder (1975) reported that, slow muscle fibres of insects have large mitochondria, however fast muscles have small mitochondria. In many insect skeletal muscles the mitochondria are smaller than those of the flight muscles and occur in pairs, the flight muscles are much more aerobic than skeletal muscles (Elder, 1975). The muscles studied here are a mixture of fibres with different sizes and shapes of mitochondria. The size of mitochondria within the flexor muscles is relatively much smaller than those of other insects e.g. much smaller than those of the muscles of the cockroach. Therefore the difference between mitochondrial sizes within the muscles of E. calcarata is supporting that, the muscles are a mixture of fibres with different oxidative metabolism, with predominantly less aerobic fibres than other insect muscles. The different mitochondrial shapes within the muscles can also be an evidence that, the muscles are a mixture of less and more aerobic fibres.

The mitochondrial sizes within the muscle of the male are significantly different from those within the female ($p < 0.001$), the muscle of the female has larger mitochondria than the male which again suggests that the female has more aerobic fibres. The population in dorsal region of the male is significantly different ($p < 0.001$) from that of the ventral region, but the dorsal region population of the female is not significantly different ($p = 0.479$) from that of the ventral

region. This may support that the muscle of the female has more uniform fibres population and distribution, with more slower fibres.

One of the most significant ultrastructural differences between muscle fibres which can predict physiological fibre types is the amount of sarcoplasmic reticulum (SR). The different amount of SR may change the rate of release and sequestration of calcium, which is essential for muscle contraction. The rate of release and sequestration of calcium in fibres with less SR will be slower than in fibres with larger amount of SR (Cochrane at al, 1972).

The high correlation between the amount of SR and the speed of crustacean and insect muscle fibres has been demonstrated by many authors. SR is much more developed in fast fibres than in slow ones (Cochrane et al, 1972; Cullen, 1975; Edwards et al, 1956; Elder, 1971; Fahrenbach, 1964, 1967; Franzini-Armstrong, 1970; Hagopian and Spiro, 1967; Hoyle, 1978; Huddart and Oates, 1970; Hughes, 1965; Jahromi and Atwood, 1967; Peachey, 1965; Philpott and Goldstein, 1967; Prosser, 1973; Smith, 1961, 1962, 1966, 1984; Smith and Sacktor, 1970; Walcott and Burrows, 1969). There are highly significant differences between SR areas of some parts and others within the muscles of both the male and the female ($p < 0.001$), the muscles are heterogeneous. There are significantly different between most parts within the male and between 50% parts within the female ($p=0.05$). The range of SR

area of fibres within the muscles is great, 3.87%-25.3% for the male and 6.75%-19.05% for the female. Cochrane et al (1972) compared tonic and phasic fibres of the large extensor tibialis muscle and the retractor unguis muscle of the metathoracic leg of the locust Schistocerca gregaria. In the tonic fibres of the extensor tibialis muscle SR is 1.1% of fibre volume, and in phasic ones it is 6.8% of fibre volume. In the retractor unguis muscle of the locust, which is fast, SR occupies 19% of fibres volume. Morgan and Stokes (1979) examined two mesocoxal muscles Periplaneta americana, the posterior coxal depressor muscle (muscle 136) and the coxal branch of the main depressor group (muscle 135). Muscle 136 is fast and SR is 33.9% of the muscle fibres volume. The ventral fibres of 135d which are also fast have 26.6% SR. In the dorsal fibres of 135d which are slow SR occupies only 13.6% of fibres volume. By comparison, the muscles studied here contain fibres with much higher density of SR than the extensor muscle of Schistocerca gregaria which may suggest that the muscles are much faster but the majority of fibres are slower than the retractor unguis muscle of the locust and slower than mesocoxal muscles of the cockroach. Huddart and Oates (1970) observed that, in the metathoracic extensor tibialis muscle of the locust Locusta migratoria migratorioides, the SR which surrounded each myofibril is as much as 15% to 20% of the cross sectional area of the muscle fibres. They stated that, this amount is typical for fast muscle of insects and crustaceans. The SR occupies about 18% of main jumping muscle fibres of the flea Xenopsylla cheopis

(Cullen, 1975). The majority of muscle fibres from E. calcarata have much less SR than those of L. migratoria migratorioides and the flea. Therefore the muscle from the male and female of E. calcarata which is a mixture of fibres with significantly smaller and larger area of SR, can be further evidence that the muscles are a mixture of faster and slower fibres. The flexor tibialis muscles contain relatively much less SR than other fast insect muscles which suggests the muscles contain relatively much slower fibres.

The SR areas within the muscle of the male is significantly different from those within the female ($p=0.009$), the male has larger SR areas than the female. The parts which are significantly different from each other are more in the muscle of male than those within the female. The male has greater range of SR area. The population in the dorsal region of the muscles of both the male and the female are significantly different from that of the ventral region in the muscles. The differences between the mean SR areas in the dorsal and ventral regions in the male was higher than that of the female. These indicate that the fibres population and distribution within the muscle of the female is more uniform than in the male. The muscle of the female has a smaller area of SR than that of the male, this may suggest that the male contains relatively more faster fibres.

Fast fibres have more dyads and have a better developed SR than slow ones. Moreover the SR perfectly surrounds the

myofibrils in fast fibres (Atwood, 1972; Cochrane et al, 1972; Fahrenbach, 1967; Franzini-Armstrong, 1970; Hagopian, 1966; Hoyle, 1978; Huddart and Oates, 1970; Jahromi and Atwood, 1967, 1969a; Smith, 1966). The junction between SR and T tubular-system (TTS) which forms dyads is typical of insect muscles (Smith, 1966). Atwood (1972) stated that in crustacean muscles two or more dyads commonly exist around each myofibril, in the dyad rich region. Huddart and Oates (1970) compared the metathoracic extensor tibialis muscle of the locust, Locusta migratoria migratorioides, and the prothoracic flexor tibialis muscle of the stick insect, Carausius morosus. The former muscle has a much more expanded SR and more frequent dyads per unit area than latter and the stick insect muscle is considerably slower than the locust. In tonic abdominal extensor muscles of crayfish Procambarus clarki and Orconectes virilis, which have long sarcomeres dyads are fewer than in the fast ones which have short sarcomeres (Jahromi and Atwood, 1967). In the muscle fibres observed here each straplike, polygonal or irregular myofibril is surrounded by one to three rows of sarcoplasmic reticulum and one or several dyads. Thus the different amount of dyads can be additional evidence that the muscles are a mixture of fibre types; slow, intermediate and fast fibres.

From the literature on the ultrastructure of the muscles one can conclude that, the sarcomere length is strongly correlated with fibre types in invertebrates. Sarcomere length is more important to determine fibre types than the ratio of thin to

thick filament e.g. the different coxal muscles of the cockroach have similar sarcomere lengths but different ratios, 3:1 and 6:1 (Jahromi and Atwood, 1969b), yet these muscles have similar contraction speed and produce similar amount of tension per cross sectional area (Usherwood, 1962). It can also be concluded that, the amount of sarcoplasmic reticulum (Cochrane et al, 1972; Huddart and Oates, 1970), the amount of mitochondria (Elder, 1975) and thick filaments number (Jahromi and Atwood, 1969a) are strongly correlated with fibre types. Whereas the cross sectional area of fibres (Stokes, 1987) and absolute number of thin filaments (Jahromi and Atwood, 1969a) are less significant to predict muscle fibre types.

The above comparison of the amount of mitochondria and average size of mitochondria of fibres within the muscle of E. calcarata with those of crustacean and other insect muscles shows that, the muscle of E. calcarata contains much less aerobic fibres than muscles of crustacean and other insects, the comparison for the thick filaments number per μm^2 shows that the muscle contains much faster contraction fibres than crustacean and other insects. Whereas by comparison of amount of SR of the muscle fibres studied here with those of other arthropods shows that, the muscle of E. calcarata contains fibres with much slower contraction and relaxation; the ratio of thin to thick filaments shows that the muscle contains slower contraction fibres than crustacean and with very similar properties to other insect muscles; the cross

sectional area of fibres predicts that the flexor tibialis muscle contain much slower fibres than crustaceans and that the muscle contains fibres with similar contraction velocity or faster than other insects; and the sarcomere lengths show that the flexor tibialis muscle contains a majority of intermediately fast fibres.

All the ultrastructural criteria show that the population of fibres within the muscles of both the male and the female is heterogeneous, and that the differences between some parts within the both muscles are highly significant from others ($p < 0.001$). All the criteria (with exception of mitochondrial number) also show that most parts within the muscles of the male and the female are significantly different from each other ($p=0.05$). These suggest that the ultrastructural properties of fibres of some parts within the muscles are significantly different from others. Therefore from these results, it can be predicted that the physiological properties of some parts are different from those of other parts i.e. the muscle contain parts with; fast contraction and rapid fatigue fibres, with fast contraction fatigue resistant fibres and parts with slow contraction and fatigue resistant fibres. The range and STD of the all ultrastructural parameters within the muscles and within some individual parts are great. These predict that the individual parts contain fibres with different physiological properties.

The all ultrastructural criteria (with exception of the thin

to thick filaments ratio) show that the population within the muscle of the male is highly significantly different from the population within the female (Table 20). The mean sarcomere length is longer and the mean thick filament number is lower within the male than those within the female. These may suggest that the muscle of the female contains more faster fibres. The means of fibre cross sectional area and SR area within the muscle of the male are larger than within the female and the means of thin filament number and of area, number and size of mitochondria within the muscle of the male are smaller than that of the female, which would predict that the muscle of the male contains more faster and less aerobic fibres than that of the female. Therefore by comparison of all ultrastructural parameters of the male and those of the female, it can be concluded that the muscle of the male should be contained more faster and less aerobic fibres.

The ANOVA test for all the ultrastructural parameters within the muscle of the male (with exception of thick filaments number) show that the population of the dorsal region is highly significantly different from that of the ventral region i.e. the differences between some dorsal parts and some ventral parts are highly significant, this also indicates that some of the parts of either region are different from all of the parts of the other region ($p < 0.001$). The means of all parameters with exception of the sarcomere length and cross section area of fibres predict that the dorsal region contains more slower and more aerobic fibres than the ventral region.

The dorsal region has a higher mean filament ratio, higher mean thin filament number, lower mean thick filament number, smaller mean of SR area, larger mean area, size and higher mean number of mitochondria, than the ventral region.

All the ultrastructural parameters (with exception of thin filaments number and mitochondrial size) show that the population of the dorsal region within the female is significantly different from that of the ventral region. Some dorsal parts are significantly different from some ventral parts, this also indicates that some of the parts of either region are significantly different from all of the parts of the other region (table 20). The means of some of the parameters; mean of sarcomere lengths and the mean fibres cross sectional areas predict that the dorsal region contains more faster fibres than the ventral region. The means of SR areas, thick filaments number and filaments ratio predict that the dorsal region contains more slower fibres than the ventral region. The area and number of mitochondria predict that the dorsal region contains less aerobic fibres. The differences between the means in dorsal region and those in the ventral region, for most parameters, within the female are lower than those in the male and the levels of probability for differences between the dorsal region and the ventral region in the female were similar or higher than those in the male (with exception of thick filaments number). The range and STD of the all ultrastructural parameters (with exception of number of mitochondria) within the male are greater than those

of the female. These suggest that the muscles are a mixture of physiological fibre types, with predominantly faster fibres. The muscle of the female is more uniform, and contains more slower fibres. This correlates with the histochemical evidence which also suggests that the muscles are heterogeneous. The histochemical tests confirm that the muscles are a mixture of physiological fibre types with predominantly fast fibres and that the muscle of the female contain more uniform distribution and population fibres with more slower fibres than the muscle of the male, and that the slower fibres in the muscle of the male are more frequent in dorsal region.

All the ultrastructural results within the muscles of both the male and the female showed that the muscles are heterogeneous, some parts within the muscles are highly significantly different from others ($p < 0.001$). All the results (with the exception of mitochondrial number in the male) showed that most parts are significantly different from each other ($p=0.05$). From the knowledge of specific relationships between ultrastructural and physiological properties of muscle fibres, the physiological fibre types can be predicted from the ultrastructural results. Having these objects in mind the muscle parts were ranked from the fastest to the slowest, according to the ultrastructural results. These results show that the parts with specific fibre types are not located in specific regions of the muscles. Most ultrastructural results show that the majority of the muscle parts from the male with

slower fibres are located in dorsal region (table 31). Most parts which have fibres with smaller mean area of SR, lower mean density of myosin filaments, higher mean thin to thick filament ratio and larger mean area and number of mitochondria, typical of parts with slow contraction and fatigue resistance fibres (more aerobic) are located dorsally. However these parts contain fibres with shorter mean sarcomeres and larger mean cross sectional area which is characteristic of parts which have faster fibres. It can, therefore, be concluded that these parts should be contained intermediate slow fibres. Some dorsal parts of the male have fibres with mean shorter sarcomeres, larger mean cross sectional area, high density of myosin filament, and high density of mitochondria (Table 31). These should be parts which contain fibres with fast contraction and fatigue resistance.

Some ventral parts have fibres with larger mean sarcoplasmic reticulum area, lower mean ratio of thin to thick filament, higher mean myosin density and lower mean mitochondrial density (table 31), which suggests that these parts contain fibres with fast contracting and fatigue rapidly. Some muscle parts from the male may contain fibres with slow contraction and fast fatigue e.g. VPP, these parts contain smaller mean cross sectional area, higher mean ratio, lower mean myosin density, which predicts that these parts contain fibres to be slow contraction, but with lower mean number and smaller mean area of mitochondria which is characteristic of the parts

Table 31: Showing the position of the 12 muscle parts for the male (upper table) and the female (lower table) of E. calcarata, according to the results of each ultrastructural parameters. Number (1) indicate the fastest and number (12) the slowest. SL= sarcomere length, Fib area=cross sectional area of fibres, Fil ratio= filament ratio, Myosin dens=myosin filament density, Mito area=area of mitochondria, Mito No=number of mitochondria, SR area=area of sarcoplasmic reticulum area.

	"fast"	DDA	DDP	DMA	DMP	DPA	DPP	VDA	VDP	VMA	VMP	VPA	VPP	"slow"
SL	short	1	2	6	7	10	3	8	9	11	12	5	4	long
Fib Area	large	7	1	2	4	3	5	6	8	10	11	9	12	small
Fil ratio	low	10	11	8	9	7	6	5	2	3	1	4	12	high
Myosin dens	high	12	9	7	8	3	2	11	6	4	1	5	10	low
Mito area	small	8	11	12	6	9	10	2	4	3	1	7	5	large
Mito No	low	7	8	11	10	12	6	1	9	4	5	2	3	high
SR area	large	12	9	2	8	10	11	4	6	7	5	3	1	small

	"fast"	DDA	DDP	DMA	DMP	DPA	DPP	VDA	VDP	VMA	VMP	VPA	VPP	"slow"
SL	short	2	9	4	7	3	10	8	5	6	12	1	11	long
Fib Area	large	3	10	11	9	2	7	6	1	12	4	5	8	small
Fil ratio	low	12	3	9	4	6	10	2	11	5	8	7	1	high
Myosin dens	high	12	8	11	7	2	5	1	10	9	4	6	3	low
Mito area	small	3	2	4	5	9	1	12	11	6	8	10	7	large
Mito No	low	2	1	3	7	5	4	8	6	10	9	12	11	high
SR area	large	12	10	9	8	4	3	5	11	6	1	2	7	small

which contain fast fatigue fibres. The results of ultrastructural examinations on the muscle of the female (table 31) show that, the distribution of parts with faster and slower fibres are more uniform than those of the male e.g. all dorsal parts within the muscle of the male are with medium and high ratios, however in the female about 50% of the dorsal parts are with lower and 50% with higher ratios. The ultrastructural results correspond with the histochemical results which showed that the distribution of fibres with darker and lighter staining for ATPase and NADH-TRase of the muscle from the female is more uniform than that of the male, the muscle of the female contain more slower fibres.

The results of all ultrastructural parameters also show that within the ventral and dorsal regions of both the male and the female some parts are significantly different from one another ($p=0.05$). Both the ventral and dorsal regions of the male and the female have parts with different physiological fibre types (table 31). The results also showed that the range of all criteria in the individual parts and whole muscle was great. Therefore the muscles of both the male and female should be contained a range of physiological fibre types, fast contraction-fast fatigue, slow contraction-fatigue resistant, fast contraction-fatigue resistant and few fibres with slow contraction-fast fatigue.

4.3. MUSCLE PHYSIOLOGY

Ultrastructural analysis permits us to examine the range of fibre types and predict the physiological performance of the muscle but can not predict how the muscle may be used by the animal. For this reason it was necessary to examine the physiology of the muscle in the intact animal so that the performance of the muscle could be analysed in the context of the behaviour.

Josephson (1985) states that, the most significant functional capacity of the muscle is its capability to shorten against a load and thus to do work. There are many studies of tension produced by whole muscle or muscle fibres at fixed length, where muscle contracts with no external work done and there are also many studies on mechanical work output by active animals. Despite its importance, work production by muscle is infrequently studied. Therefore there is a need for further studies on capacity of the muscle to do work, produce force and shorten against load.

The metathoracic flexor tibialis muscles of the adult male and female E. calcarata were physiologically analysed, in order to compare differences between physiological responses of the specialized legs and unspecialized ones. The metathoracic legs of the male were compared to those of the female, since they are homologous but have significant morphological and

functional differences. The metathoracic legs of the male are larger, being used for defensive strike behaviour in addition to locomotion, whereas those of the female are much less developed and have a locomotory function primarily.

The responses examined were behavioural as the intact insects were stimulated by noxious (electrical) stimulation in order to obtain maximal uniform evoked behavioural responses for all experiments.

Some previous observations show that the correlation between increase in muscle load and decrease in velocity of muscles. The load versus velocity curves show that even minimal loads, reduce the muscle velocity and when load is maximal the velocity is zero (Close, 1964; Fenn and Marsh, 1935; Hill, 1950, 1958; Huddart, 1975; Josephson, 1984; Johnston and Wokoma, 1986; Podolsky, 1960; Prosser, 1973; Woledge, 1968). Load versus velocity curves of many muscles show that the velocity of the muscles decrease quicker during initial increase in load than with increasing relatively heavier loads (Else and Bennett, 1987; Hill, 1970; Johnston et al, 1985; Marsh and Bennett, 1986; Ramsey, 1965; Wells, 1965). When the male E. calcarata muscle load was increased from 139.2 g to 278.4 g the velocity decreased 15.7% of the maximum measured velocity but when the load was increased from 1531.2 g to 1670.4 g the velocity only decreased 2.9% of the maximum measured velocity, therefore the load-velocity curve is nonlinear. Thus the correlation between increase in load and

decrease in velocity for the muscles studied here is similar to previous observations. The velocity of the flexor tibialis muscle of the female decreases in a similar manner with increase in load, however the percentage decrease in velocity of the female is much greater, for any increase in load, than that of the male i.e. the male can sustain much heavier loads and shorten with higher velocity than the female.

Many fast muscles exhibit less curvature than slow muscles in their load–velocity plots (Josephson, 1984; Marsh and Bennett, 1986; Woledge, 1968). Comparison of the load–velocity curves of muscles from the male and female E. calcarata, shows similar correlation between the velocity of the muscles and their degree of curvature, the muscle of the male is faster and has lower degree of load–velocity curvature than that of the female.

The flexor tibialis muscle of E. calcarata particularly of the male produces relatively high power output and they are considered to be relatively fast. The maximum velocity, at zero load, estimated by extrapolation of the curve of load versus velocity of the muscle from the male is about 22.5 $mm.s^{-1}$ and that of the female is 18.0 $mm.s^{-1}$. The velocity of the muscle of the male reaches zero at a maximum muscle load of 2500 g, estimated by extrapolation of the load–velocity curve, and that of the female at the maximum load of 1500 g. The flexor tibialis muscles of the male and female E. calcarata are faster than flight muscle of the locust

Schistocerca gregaria, with maximum velocity of 10 $mm.s^{-1}$ (Buchthal et al, 1957) and slower than the metathoracic tergocoxal muscles of two tettigoniid insects Neoconocephalus robustus and N. triops, which are comparable to very fast muscle such as the extensor digitorum longus of mouse (Josephson, 1984). As mentioned later, the muscles studied here produce more tension per cross sectional area than the prothoracic flexor tibialis muscle of the stick insect, Carausius morosus and the extensor tibialis muscle of the locust. Therefore the muscles of E. calcarata are relatively powerful muscles and have higher capability to do work. Comparison of the velocity and force produced by the flexor tibialis muscles of the male and the female, shows that the muscle of the male is much faster and generates more force than that of the female. The higher velocity and more force generated by the male relates to the evoked behavioural function of metathoracic legs of the male. i.e. the male requires to be fast and produce high power output to impact predator to the main femur spine and inflict maximum damage.

The performance of muscles may be influenced by the limb dimensions (Gabriel, 1985; Gray and Mill, 1985; Huddart and Oates, 1970; Myers and Steudel, 1985). Huddart and Oates (1970) examined the prothoracic flexor tibialis muscle of Carausius morosus and metathoracic extensor tibialis muscle of Locusta migratoria migratorioides and found that the muscle of the stick insect is slower than that of the locust. They suggest that the slowness of the former muscle may depend on

the narrowness of the leg and small interfibre spaces in the muscle. Velocity of the male E. calcarata is much higher than that of the female, possibly because of the enlarged metathoracic femur of the male compared with the correspondingly narrow femur of the female. The enlarged femur of the male contains larger muscles than that of the female.

The correlation between muscle load and work done by the muscles studied here is similar to several previous observations (Buchthal et al, 1957; Josephson, 1985; Prosser, 1973). The relationship between load sustained and work done during initial contraction by both muscles of the male and female produce bell shaped curves. The work done reaches a peak at an intermediate load of 974.4 g for the male, and the work done for the female reaches a peak earlier than the male, at 407.2 g. At the two ends, at zero load and maximum load, estimated by extrapolation to be 2450 g for the male and 1500 g for the female, the work done is zero. The work done during delayed contraction by the male however reaches a peak at a load of 1670.4 g which is close to heaviest load at which the work done was measured. The pattern of work done during delayed contraction by the muscle of the female is similar to that of the male.

Buchthal et al (1957) and Woledge (1968) show that, work done by insect and vertebrate muscles increases quicker during initial increase in load i.e. loads lighter than the optimum load and decreases slower with increasing relatively heavier

load. The maximum work done by the muscle of the male E. calcarata occurs at 39% of the maximum load, at which the work done reaches zero and that of the female occurs at 27% of the maximum load, at which its work done reaches zero. Buchthal et al (1957) show that the power of the flight muscle from the locust is optimal from 0.3 to 0.4 of maximum load. Therefore for E. calcarata, the correlation between increase in work done during initial increase in load (lighter than optimum load) and decrease in the work done with increasing relatively heavier loads (heavier than optimum load) is similar to previous findings.

The maximum work done by the muscles of the male and the female during initial contraction, 690.0 g.mm by the male and 384.3 g.mm by the female, and during delayed contraction, 1907.6 g.mm by the male and 739.9 g.mm by the female, are much greater than some insect flight muscles (Buchthal, 1957; Josephson, 1985). I am not aware of any study on insect limb muscle which shows the external work done, when muscle sustained a load. The maximum work done during initial and delayed contraction by the muscle of the male is greater than that of the female. The differences between the male and female can be related to the different behavioural function of the metathoracic legs.

The relationship between work done and velocity of the flexor tibialis muscle is similar to previous findings (Buchthal, 1957; Else and Bennett, 1987; Hill, 1950; Langfeld et al,

1989). When the velocity of the muscles from E. calcarata was very low or very high the work done fell strongly towards zero. The load at which maximum work was done corresponds to a velocity of 0.35 times the maximum velocity for the male and 0.42 times of the maximum velocity for the female. Buchthal et al (1957) shows that, the maximum work done by the flight muscle of the locust occurs at about 0.40 time of maximum velocity at zero load.

The maximum measured power output and work output of the flexor tibialis are greater than those of some insect muscles (Gray and Mill, 1983; Josephson, 1985) and similar or lower than others (Bennet-Clark, 1975; Buchthal et al, 1957; Weis-Fogh, 1956). The maximum measured power output of the flexor tibialis muscle from the male, 41.17 $W.Kg^{-1}$, and that of the female, 52.01 $W.Kg^{-1}$ and maximum measured work output of the male, 3.4 $J.Kg^{-1}$ and that of the female, 4.33 $J.Kg^{-1}$, are much greater than that of the first tergocoxal muscle of the tettigoniid Neoconocephalus triops. The average work output during isotonic twitches of the metathoracic, flight muscle, of the tettigoniid is 1.3 $J.Kg^{-1}$ (Josephson, 1985). The specific mechanical power output of flight muscle can be near the maximum of which striated muscle is capable. The isotonic twitch work produced by flight muscles of locust is up to 4.5 $J.Kg^{-1}$ (Buchthal et al, 1957). Maximum power output during the mantid strike from the extensor and flexor tibialis muscles of the prothoracic leg of the praying mantid Heirodula membranacea are 6 $W.Kg^{-1}$ and 27 $W.Kg^{-1}$ respectively (Gray and

Mill, 1983). The work output of the E. calcarata flexor tibialis is similar to the work output of the flight muscle of the locust and the power output is greater than flexor and extensor muscles of the praying mantid. Thus the muscles studied here are relatively powerful and have a high efficiency to do work.

Velocity of muscles, their power and efficiency may be indirectly correlated e.g. the maximum velocity of the anterior parts of the extensor iliotibialis of the salamander, Ambystoma tigrinum nebulosum at zero force is about 20 $mm.s^{-1}$. Maximum power output of the salamander is approximately 125 $W.Kg^{-1}$ (Else and Bennett, 1987). The muscle of the male E. calcarata produces less power output than the salamander but the velocity of the male at zero force is higher. Moreover the muscles of the male and the female are slower but more powerful than the flexor and extensor tibialis muscle of the prothoracic leg of the praying mantid (Gray and Mill, 1983). The physiology results on the muscle of E. calcarata also show that the muscle is slower than some other very fast insect muscles, but that it produces much more power output.

The flexor tibialis of the E. calcarata sustained much heavier loads and produced more tension than other insects (Buchthal et al, 1957; Hoyle, 1955; Josephson, 1984; Wood, 1958). The maximum load sustained by the muscles studied here, 1948.8 g for the male and 1425.2 g for the female, is much greater than that of the prothoracic flexor tibialis muscle of Carausius

morosus which lifts only 2 g (Wood, 1958). The calculated maximum tension produced by both the muscles studied here, 1,077 $g.cm^{-2}$ and 4182 $g.g^{-1}$ for the male; 1,336 $g.cm^{-2}$ and 5770 $g.g^{-1}$ for the female, are greater than maximum tension produced by the extensor tibialis muscle of the locust, 1,000 $g.cm^{-2}$ and twitch tension of 2500 $g.g^{-1}$, which is more than the tension produced by corresponding muscle of the frog, 1000 g.g-1 (Hoyle, 1955) and are much greater than that of flexor tibialis muscle of the Carausius morosus. 800 $g.cm^{-2}$ (Wood, 1958). Therefore the flexor tibialis muscles studied here are relatively very powerful muscles.

Muscles with high velocity may develop less tension than slow muscles (Josephson, 1984; Mendelson, 1969; Prosser, 1973). The calculated tension produced by the muscle of the female whilst undergoing work, 5,770 $g.g^{-1}$ and 1,336 $g.cm^{-2}$, is higher than that of the male, 4,182 $g.g^{-1}$ and 1,077 $g.cm^{-2}$ and the muscle of the female is slower. The maximum velocity of both muscles of the male and female are lower than the metathoracic and mesothoracic muscles of both N. triops and N. robustus (Josephson, 1984) and produce higher maximum tension than the muscles of N. triops and N. robustus . The produced tension by N. triops and N. robustus is similar to the range of tension produced by most striated muscles (Close, 1972). Therefore the muscles of the male and female E. calcarata are relatively slower but generate more tension.

The force produced by the flexor tibialis muscle of E.

calcarata, 152.42 mN for the male and 30.6 mN for the female, are much higher than the force exerted by the flexor and extensor tibialis muscles of the prothoracic and metathoracic legs from the stick insect, Cuniculina impigra (Bässler, 1984, 1988) and also higher than force produced by the flight muscle of the locust (Buchthal et al, 1957). The load sustained by the flexor tibialis muscle of E. calcarata is about 1000 times of the load lifted by the prothoracic flexor tibialis muscle in Carausius morosus (Wood, 1958). Comparison of the force produced by the muscles studied here with those of Cuniculina impigra and the locust shows that the flexor tibialis of E. calcarata is a relatively powerful muscle. The muscle of the male produced much more force than the female. These differences can be related to the different evoked behavioural functions of the metathoracic legs of the male and the female.

The force and velocity of muscles and of muscle fibres may be directly correlated (Johnston and Salamonski, 1984; Johnston et al, 1985; Wells, 1965). Wells (1965) has shown that, the maximum velocities of soleus and anterior tibialis muscles of albino rat are, 54 $mm.s^{-1}$ and 144 $mm.s^{-1}$ respectively and the faster anterior tibialis exerts approximately 5 times the isometric force of the soleus muscle. The flexor tibialis muscle of the male E. calcarata is faster than that of the female, maximum measured velocity for the male being 17.37 $mm.s^{-1}$, whereas that of the female is 11.59 $mm.s^{-1}$, and the male produced a maximum force of about 5 times that of the female. Therefore the correlation between the force produced

by the muscles and their velocity is similar to previous observations.

The relationship between the femur-tibia joint angle and tension produced by the flexor tibial muscle, observed here, is similar to that of previous findings. As the flexion angle is decreased from total extension the tension increases until reaches a peak at the femur-tibia joint angle of 1.22 rad, when the joint angle is close to the angle of the normal standing position on a flat surface. With further decreases in the flexion angle, the tension also decreases. Prosser (1973) and Huddart (1975) stated that, in general as the muscle length is increased tension increases until it reaches to maximum followed by a decline at longer lengths. Maximal tension occurs at a length, which for the majority of striated muscles, is near the normal resting length in the body. Therefore total isometric tension is a function of muscle length and the muscle length is changed as femur-tibia joint angle changes.

The physiological results show that the flexor tibialis muscle is a relatively fast and produces high power output. The muscle of the male, however, is much faster and produces more power output than that of the female, the maximum force produced by the muscle of the male is 5 times that of the female and the work done by the male is much greater than that of the female. The muscle of the male sustained much heavier loads than that of the female. The work done, when the loads

are increased, by the muscle of the female reaches peak much earlier than that of the male i.e. the muscle of the male can sustain much heavier loads and do much more work for a longer time and produced much more power than the female. The velocity of the female also falls much quicker, fatigues much faster, than the male. The differences between the physiological properties of the muscle of the male and the female must relate to their different behavioural functions. The specialized metathoracic legs of the male being used for the defensive strike as well as locomotion, whereas the legs of the female are used for locomotion primarily.

Comparison of physiological properties of the flexor muscle of the E. calcarata, with other insect muscles showed that the E. calcarata, produces much more power output than other insects. The muscle can sustain much heavier loads than other insect muscles. Although the muscles are slower than some other very fast muscle e.g. slower than praying mantid, the muscle of the E. calcarata produces much more power and work output. E. calcarata also produces more power and work output than some flight muscles, the most active muscles, the power outputs of which are near the maximum for striated muscle to produce. The force and tension produced by the E. calcarata particularly that of the male are relatively much higher than those of other insect muscles. The high power of the muscle of the E. calcarata again suggests that its physiological properties are related to the behavioural function of the insect. The muscle is not very fast and it is not required to

be very fast e.g. as praying mantid, which the mantid uses its speed for prey capture, but it should be able to produce high power output, in order to keep the predator between femur-tibia joint and impale the object on to the main femur spine by flexion of the tibia.

The histochemical and ultrastructural results showed that the muscles are a mixture of physiological fibre types with predominantly fast fibres and that the muscle of the male contains more fast fibres than the female. The physiological results also showed that the muscles contract relatively fast during initial contraction and can remain in contraction for quite long time, more than 30 sec, during delayed contraction and that the muscle of the male is faster and generate more force and power output then the female. The muscle of the male requires to be fast, to flex the tibia rapidly to catch an attacker and also to be able to produce higher power, to do considerable damage to the predator during tibial flexion. The higher speed of the flexor tibialis from the male gives the insect greater ability to catch an attacking predator and the greater produced power output by the muscle gives the male greater ability to inflict damage on predator. Therefore the mixed properties of the muscle predict that the muscle fibres will be recruited by the nervous system in a pattern suited to the behaviour. Initial, reflex, contraction should primarily recruit fast fibres, whereas the delayed, voluntary, contraction will use a mixed population of intermediate and slow fibres.

When the load sustained by the muscle was increased from the lightest to the heaviest, which the muscle sustained, the initial contraction diminished to zero. Whereas the delayed contraction diminished by about 50% i.e. the muscle is able to remain in contraction during delayed contraction and do much more work by recruitment of intermediate and slow fibres which fatigue slowly. When the insect was stimulated repeatedly for a long time the result showed that the tension decreases with time gradually. Again this is corresponds with ultrastructural and histochemical results which suggest that the muscle contains fast, intermediate and slow fibres i.e. fibres with different contraction and fatigue rate. The diminution of isometric tension within the first bursts, observed during repetitive stimulation at 1 Hz is also related to faster fatigue of fast fibres. The muscle also produces tension after a long period of stimulation which is related to fatigue resistance of slow muscle fibres.

4.4. Mechanical properties of the leg and the muscle

The defensive strike of the male E. calcarata is performed by the metathoracic flexor tibialis muscle and is limited by the physiological properties of the muscle and mechanical properties of the leg. The defensive strike correlates with the structure of the legs and the physiological response of the flexor tibialis muscle. Any predator that attacks the insect, if caught would be grasped by the metathoracic legs and impaled on the main femoral spine by rapid initial contraction of the flexor tibialis muscle, then held within the femur-tibia joint angle, between the femur and tibial spines for a long time, more than 30 sec, with the high power produced during delayed contraction. Thus the male E. calcarata is capable of doing considerable damage.

The maximum extension of the tibia of E. calcarata, 2.45 rad, is similar to that of other insects legs. The angle of the femur-tibia joint in the jumping leg of the rabbit flea Spilopsyllus cuniculus can extend to 2.27 rad (Bennet-Clark and Lucey, 1967). Bennet-Clark in (1975) reported that, the full extension of the metathoracic leg of the locust is between 2.44 rad to 2.61 rad. The degree of extension of the tibia of E. calcarata, enables the insect to catch a relatively large object. The size of the largest cylindrical object which the insect is able to impale on top of the main femoral spine and hold between the main femur and tibial spine, during the defensive strike, is about 13 mm in diameter

at flexion angle of 1.8 rad, this is large enough to be the beak of a bird, the head of a lizard or the mouth of a small mammal. Clail (1988) observed, the males in culture condition fight with each other particularly when they are mixed with females, during competition for the female. The insect also would be able to catch the leg of another male during fighting.

There are some observations which show the correlation between the structure and mechanical properties of insect or crustacean legs, muscles and their behavioural functions. The prothoracic legs of the praying mantid Heirodula membranacea (Gray and Mill, 1985) are greatly adapted for predation, being long and massive, both the femur and tibia are heavily armed with spines and the joints are very mobile. Prey is captured rapidly and precisely by directed movement of the legs. After the strike the praying mantid holds the prey by contraction of the large flexor tibialis muscle.

The relationship between the morphology of the metathoracic legs of jumping insects and their function has been also observed by some authors. The metathoracic leg articulation of the locust Schistocerca gregaria is specialized for jumping (Bennet-Clark, 1975) and allows the locust to extend the leg to a high degree, 2.44-2.62 rad. Gabriel (1985) observed that the significant difference between the jumping of the adult locust Schistocerca gregaria forskal and hoppers is due to the change in length of the metathoracic leg and in extensor

muscle mass. The metathoracic femur is more developed in the adult locust and it accommodates a larger extensor muscle. Therefore, relatively more force and energy may be available for use in a jump. In Xenopsylla cheopisa, a pulicoid flea, the mesocoxa and second pair of legs are considerably reduced. However the metacoxa and the metathoracic leg are well developed and enlarged. This enables the flea to jump properly, which is performed by rapid and powerful extension of the metathoracic legs (Rothschild and Schlein, 1975).

The metathoracic femur of the male E. calcarata is well developed and is relatively very large. The femur bears 4 spines of which one is very large, 6.20±0.11 mm, and its walls are made of strong cuticle. Any predator that attacks the insect if caught will be impaled on the top of main femoral spine by high force produced by the muscle. Bedford (1975) suggested that, the behavioural function of this behaviour in E. calcarata is defensive i.e. the behaviour is given when the insect is molested and is not seen if the insect is left undisturbed. However Clail (1988) and Hsiung (1987) observed that the males, particulary when they are held with females, will engage in aggressive encounters with other males. The force produced by the muscle can penetrate even the thick cuticle of the femur in another male or female. The fighting between males has been observed by Clail and Hsiung in laboratory conditions and may not be representative of E. calcarata under natural condition, in the field. It is possible that if the insect is disturbed by others in field

they just walk away. Therefore the morphology of the metathoracic leg and the physiology of the flexor tibialis muscle of E. calcarata appears to have involved as a mechanism to catch predators. Since the insects are slow moving a fast contracting muscle would not be necessary to catch another male which suggests that, this is not its primary function and supports the theory that it is an anti predator advice.

The strike by the metathoracic leg of E. calcarata, with a duration of approximately 150 msec, from full extension of the femur-tibia joint to full flexion, is slower than some very fast insects e.g. slower than strike by prothoracic leg of the praying mantids which is very fast (Copeland and Carlson, 1977; Gray and Mill, 1983; Rilling et al, 1959), with a range of 30-70 msec. However the defensive strike of E. calcarata is relatively fast and E. calcarata can maintain in flexion for a much longer time than other insects, more than 30 sec. The stick insect Carausius morosus can maintain in flexion for only 1 sec (Wood, 1958). The power output of the flexor tibialis muscle is higher than that of the flexor and extensor muscle of the praying mantid (Gray and Mill, 1983) but the velocity is lower. This lower velocity may result from the compromise of obtaining high velocity from a muscle which must retain a capability for prolonged powerful contractions.

The behavioural functions of some insects and crustaceans are performed by rapid movement of their limbs, usually by extension rather than flexion of the limbs e.g. fast extension

of metathoracic leg of the locust Schistocerca gregaria for jumping (Bennet-Clark, 1975; Gabriel, 1985) and of the 2nd thoracic leg, raptorial leg, of mantid shrimp Squilla and Hemisquilla for prey capture (Burrows, 1969). There are two possible mechanisms to produce high speed of movement these are, by rapid release of stored energy or fast twitch muscle fibres.

The rapid release of stored energy is advantageous for animals, where their behavioural function is performed by a very fast movement of their limbs, which can not be achieved by the physiological properties of the single muscle. The release of stored energy provides rapid release of power, which can produce the high velocity which is necessary for fast movement. In these animals high speed is more important than high power or the control of movement for their behavioural function. Therefore they need a mechanism to store energy and release it suddenly before the movement e.g. in the jumping of the flea (Bennet-Clark and Lucey, 1967) and of the locust (Bennet-Clark, 1975), where their behavioural function is performed by one rapid extension of the metathoracic leg. These animals use the stored energy to produce high acceleration in which case they can jump for a long distance. In the strike of the mantid shrimp (Burrows, 1969) stored energy is also released rapidly to strike the prey with high speed.

In animals where power is more important than high velocity

for their behavioural function, where greater control of movement is required and in animals whose behavioural function is performed by frequent movement of their limbs e.g. walking, the rapid release of stored energy is not required. There is no evidence that the E.calcarata uses stored energy for its behavioural function.

The flexor tibialis muscle of E.calcarata, generates a maximum force of 152.4 mN (see section 3.4.6). The force transfers through the tibia to the main femur spine, when the tibia flexes by the contraction of the flexor tibialis muscle. If we assume that the area of top of the spine is 0.2 mm^2, the insect would inflict an object on the main femur spine with pressure of $7.6x10^5$ $N.m^{-2}$. The force is high enough to penetrate the strong femoral cuticle of another male, as Clail (1988) observed in laboratory culture conditions, the males frequently penetrate femoral cuticle of other males.

The maximum calculated power output of the flexor muscle of E. calcarata (see section, 3.4.4) is much higher than that of the mean maximum power from the flexor and extensor tibialis muscles of the praying mantid, Heirodula membranacea (Gray and Mill, 1983). The maximum torque acting about the femur-tibia articulation of the metathoracic leg of E. calcarata, 9711 $dyne.cm.rad^{-1}$, is much higher than the torque acting about the femur-tibia articulation of the prothoracic legs of the mantid, about 220 $dyne.cm.rad^{-1}$. Gray and Mill (1983) stated that, the power of the mantid is in range of other insects.

It, therefore, follows that the power produced by the muscle and the torque acting about the femur-tibia articulation of the E. calcarata during defensive strike is relatively higher than other insects i.e. the E. calcarata, will catch and impale the object with relatively high rotational power on the main femur spine during tibial flexion and also hold the object between the femur-tibia joint with high power.

The moment arm of the muscle increases on closing of the joint until it reaches a peak and with further decreases in angle, the moment arm decreases until total flexion. The pattern is similar to the relationship between the moment arms of the extensor and flexor muscles acting about the prothoracic femur-tibia articulation and the femur-tibia joint angle of the praying mantid Heirodula membranacea (Gray and Mill, 1983). Gray and Mill (1983) also observed similar relationship between the joint angle and the moment arm of coxal promotor about the prothoracic-coxal articulation of the praying mantid.

The remarkable morphological differences between the male and the female E. calcarata are between their metathoracic legs. The most important adaptation of the male E. calcarata to its defensive strike, is its large metathoracic femur armed with 4 spines. The flexor muscle of the male is also much more developed than that of the female. When the physiological properties of the muscle of the male and those of the female were compared (see section, 3.4), there were significant

differences between the male and the female. The muscle of the male is faster and produces a greater power output than that of the female due to its greater size. This increase in speed and power output is important for the role of leg of the male in the defensive strike. The metathoracic leg and the flexor tibialis muscle of the male E. calcarata may also be involved in aggressive actions (Clail, 1988). In their natural habitat males come out more frequently than the females. If males are the active sex in this respect, they are more exposed to predation as a consequence than females, suggested by Robinson (1968) for the stick insect, Onctophasma martini. This could therefore have been the subject of selection pressures favouring the evolution of more lines of defence.

In conclusion, the principle behavioural functions of the metathoracic limbs of the male E. calcarata are dependent on the flexor tibialis muscle. The behavioural functions are limited by the physiological and mechanical properties of the muscle and mechanical properties of the leg. The structure of the femur-tibia articulation is such that the insect can extend its metathoracic leg to a large angle which increases the probability that the insect can catch an object. The physiological results reveal that the flexor tibialis muscle is quite fast and generates high power output. The power of flexion of the tibia for the defensive strike of the insect is more important than the high speed of flexion. Since the insect does not require to catch a predator unless a predator attacks the insect, however, it needs to be sufficiently able

to produce high power output to impale the predator on the main femoral spine. The maximum calculated power output of the flexor tibialis muscle of E. calcarata (section, 3.4.4) is relatively high. The maximum torque acting about the femur-tibia articulation of the metathoracic leg of E. calcarata, 9711 dyne.cm.rad^{-1}, is also higher than other insects. Therefore the power produced by the muscle and the torque acting about the femur-tibia articulation of the E. calcarata during the defensive strike is relatively high i.e. E. calcarata can catch and impale an object with relatively high rotational power on the main femoral spine during tibia flexion and also hold the object between the femur and tibial spines with high power.

5. SUMMARY

The histochemical and ultrastructural results showed that, the muscle of both the male and female are a mixture of physiological fibre types, with predominantly fast fibres. The muscles are composed of fibres with different staining properties for both the ATPase and NADH-TRase activities. The ANOVA test for all ultrastructural criteria showed that the fibres population within muscles were heterogeneous, some parts being significantly different from others ($p < 0.001$). The T-test for all criteria, with exception of the number of mitochondria in the male, also showed that most muscle parts were significantly different from each other ($p = 0.05$).

The ANOVA for all criteria, with exception of filament ratio, showed that the differences between the population of the male and that of the female is highly significant ($p = 0.009$ for SR area and $p < 0.001$ for others). The means of most criteria predict that the muscle of the male contains more fast fibres. The histochemical examination showed that the muscle of the male contained more fibres which stained darkly for ATPase and lightly for NADH-TR.

The muscle contraction can be divided into two phases; the initial, twitch, phase was a rapid contraction with duration of 70 to 130 msec. This was followed by a slower contraction (delayed contraction) that was much longer lasting, the

muscle may remain in contraction for more than 30 sec, and more variable than initial one. The maximum measured velocity of the muscles were, 17.37 $mm.s^{-1}$ for the male whilst sustaining a load of 139.2 g and 11.59 $mm.s^{-1}$ for the female at the load of 203.6 g. The maximum initial work done was, 690.0 g.mm by the male and 384.3 g.mm by the female. The maximum work done during delayed contraction was, 1907.6 g.mm by the male and 739.9 g.mm by the female. The maximum force produced was, 152.4 mN by the male and 30.6 mN by the female. The maximum measured power output was, 41.17 $W.Kg^{-1}$ for the male and 52.01 $W.Kg^{-1}$ for the female. The maximum measured work output by the muscles were, 3.4 $J.Kg^{-1}$ for the male and 4.33 $J.Kg^{-1}$ for the female. The muscles produce relatively high power and are considered to be relatively fast with comparison to other insect muscles. The muscle of the male is faster and generates greater power output than the female. The muscle of the male produces the highest tension at the femur-tibia joint angle of 1.22 rad. The mechanical properties of the metathoracic leg and the muscle of the male also were examined.

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