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A Comparison of Shortening and Shortening Speed in Sartorius, Gastrocnemius and Rectus Abdominis Muscles of *Uromastix hardwickii*

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=ABSTRACT=

A new method is used to record the actual shortening produced during the auxotonic activity of the sartorius (SAR), gastrocnemius (GAS) and rectus abdominis (RAB) muscles of a lizard *Uromastix*. The auxotonic twitch and tetanus records thus obtained were used for the first time to calculate the coefficient of linear shortening (COLS). This coefficient represent the relative index between change in length ($\Delta L = L_\sigma - L_i$) and tension ($\Delta P = P_\sigma - P_i$) due to shortening at the steepest rising phase of the twitch and tetanus, recorded at resting length. In addition to this, maximum shortening (S_{max}) and auxotonic tensions were also determined at resting lengths of these muscles. The COLS was found to express the speed of shortening and auxotonic tensions are suggested to be of value to express the internal architecture of SAR, GAS & RAB muscles. The results are discussed in terms of contractile and elastic elements of the muscles alongwith the importance of shortening at resting lengths in skeletal muscles.

Key Words: SAR, GAS, RAB, WLS

INTRODUCTION

When a muscle is permitted to shorten, the velocity with which it begins to shorten after stimulation, is a function of the load imposed on the muscle. This phenomenon was described as the force-velocity (PV) relation by Fenn and Marsh (1935) and later by Hill (j1938). It has been observed that the shape of the PV curve depends on the length from which shortening begins. The relation between velocity and length i.e. initial to final length has been reported by Carlson (1957) for frog sartorius and by Sonnenblick (1965) for cat myocardial papillary muscles. It was also reported that when rat gracilis anticus muscles are allowed to shorten from different initial lengths, the velocity of shortening achieved at a given length was lower for longer initial lengths (Bahler et al., 1967). They have also found that the velocity of shortening depends upon the frequency of stimulating pulses received by the muscle. On this basis they concluded that velocity of shortening in rat gracilis anticus muscle is not only a function of load and length but is also related to the time elapsed from the onset of stimulation. Earlier, Abbott and Wilkie (1953) studied the validity of PV relation independent of the initial length in the case of *Rana temporaria* (sartorius) muscles, and found that the final tension values at any length were coincident whether obtained isometrically or isotonically. Langfeld et al. (1989), performed experiments on muscle fibres from the teleost, *Myoxocephalus scorpius* and demonstrated that a less curved PV relationship yields a higher

velocity and thus a greater power output for a given load. In addition, the influence of sarcomere length and passive tension on force velocity relation was also reported in isolated frog muscle fibers (Claffin et al., 1989).

In most of the studies regarding PV relation mentioned above, the scientists have subjected this relationship for analysis by applying different loads to the muscle while measuring the speed of shortening. It is obvious that the muscle length in this relation represents one of the most important parameters that affects the shape of the extreme boundaries of PV curves. In addition, Matsumoto (1967) indicated that this relation is generally being derived only for the initial length of the muscle. However, the use of resting length of a muscle in the determination of the speed of shortening was not given to consideration. The present work was therefore carried out to find the maximum extent of shortening by using a mew method and determination of speed of shortening in terms of Coefficient of linear shortening (COLS). The COLS gives a relative index for the change produced in muscle length due to shortening to that of the tension generated. These parameters were measured for various skeletal muscles of *Uromastix*.

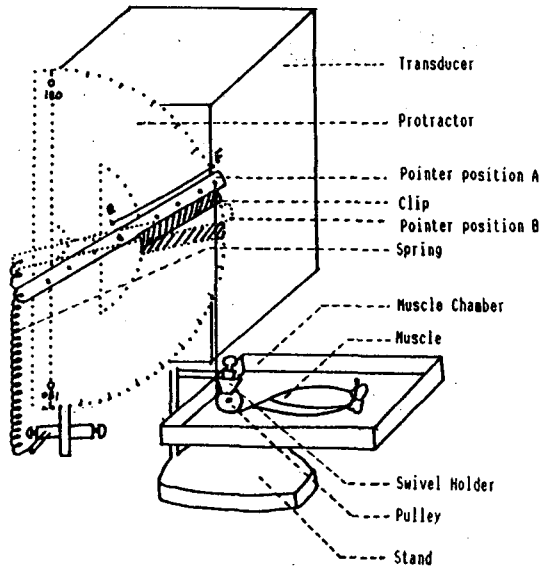


Fig. 1. Modified transducer and muscle chamber arrangement for the measurement of shortening.
Pointer position
A: Before shortening
B: After shortening

MATERIALS AND METHODS

Isolated SAR, GAS and RAB muscles of lizard, *Uromastix hardwickii* were kept in reptilian buffer (Khalil and Masseih, 1954) during experiments performed at room temperature (25±0.5℃). These muscles were stimulated at their resting lengths by square wave pulses (obtained from stimulator; Palmer Bioscience type 200) being supramaximal with a frequency of 1/sec for twitch and 80/sec for tetanus. This stimulation was applied to muscles by means of silver electrodes. The mechanical responses were recorded on a Universal Oscillograph (Harvard Apparatus) at a paper speed of 100 mm/sec. The resting length was determined by using a macromanipulator on which a transducer was installed. The calibration of this transducer, determination of resting length of the experimental muscles, measurements and calculations of twitch and tetanic tensions were similar to that used by Azeem and Shaikh (1987).

Procedure for the measurement of maximum shortening

For the measurement of maximum shortening (S_{Max}) produced in the muscle during contraction some modifications were made in auxotonic transducer as shown in Fig. 1: a) Around the pivot of the transducer a protractor was mounted. b) A light aluminum foil clip was attached at the edge of the protractor (This clip was feather weight and was movable with minimum applied force).

The foil clip was adjusted on the protractor just beneath the leaf/pointer of transducer. This position of the clip (i.e., angle from protractor) was noted prior to muscle stimulation as initial reading. Later, stimulation of the muscle rotated

the leaf/pointer that moved the aluminium foil clip on the protractor equal to the degree of its own movement which returned back to its initial position at the end of the stimulus. But the aluminium foil clip retained there represented the extent of muscle shortening and this position was noted as final reading. The difference of the initial and final readings (i.e. the angles of the initial and final position of the clip) was calculated as TanΘ by multiplying the value of the difference between thuuW4uuese two positions by $\Omega/180$. Later, For the calculation of the magnitude of maximum shortening, following formula was used:

$H = B \times Ton\Theta$

Where;

H=Magnitude of maximum shortening in the muscle (cm)

B=Length of leaf/pointer (from centre to its end where muscle was attached through a thread (Line EF in Fig. 1).

TanΘ=Difference between initial and final position of foil clip multiplied by $\Omega/180$.

Measurement of initial (P_0) and final (P_1) tensions at the steepest rising phase of twitch and tetanus

A line AB was drawn on the rising phases of twitch and tetanus and the points where the line separates from the record were marked as X and Y, as shown in Fig. 2. A perpendicular was then drawn from X towards the base line and the point where it intercepts the base line was marked as X'. Similarly, another perpendicular was drawn form Y to Y'.

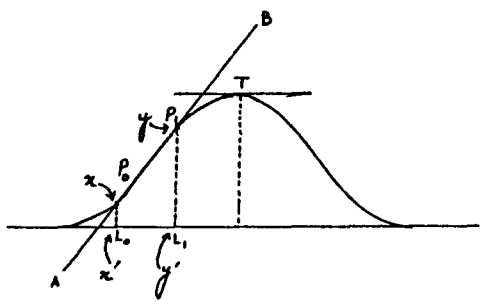


Fig. 2. Method for the Measurement of Tensions (P_0 & P_1) and Lengths (L_0 & L_1).

The line XX' was measured in millimetres and correlated with the pen deflection at the same sensitivity of the oscillograph by a known weight. This correlation gave the tension at the start of linear phase i.e. P_0 in gms. Similarly, tension at the end of linear phase i.e. P_1 was calculated by using line YY'.

Measurement of initial (L_0) and final (L_1) lengths at steepest rising phases of twitch and tetanus

L_0 and L_1, i.e. the lengths of muscle at the start and at the end of linear phase respectively, were calculated by using the following two equations:

$$L_0 = R.L. - \left(\frac{H}{T} \times P_0\right) \qquad (1)$$

$$I_1 = R.L. - \left(\frac{H}{T} \times P_1\right) \qquad (2)$$

Where;

R.L.=Resting length in cms.

H=Maximum shortening in cms.

T=Maximum tension amplitude in mms.

P_0=Tension amplitude at the start of the linear phase in mms.

P_1=Tension amplitude at the end of the linear phase in mms.

Calculations of the coefficient of linear Shortening

The coefficient of linear shortening was calculated by using a modified formula of coefficient of linear expansion with respect to temperature which is as under:

i.e., $L_1 - L_2 = K \times L_1 \times (T_2 - T_1)$

or $$K = \frac{L_1 - L_2}{L(T_2 - T_1)} \qquad (1)$$

Where;

K=Coefficient of linear expansion.

L_1=Length at T_1.

L_2=Length at T_2.

T_1=Initial temperature.

T_2=Final temperature.

The above mentioned formula represents linear expansion with respect to temperature. However, in our experiments linear shortening was involved at a particular phase of muscular activity. Since the interaction of myofilament is responsible to produce tension in the muscle which is followed by a reduction in muscle length the change in length of muscle is brought by change in tension. Hence, instead of temperature we used tension in the formula as shown below:

$$K = \frac{L_1 - L_0}{L_1(P_1 - P_0)} \qquad (2)$$

Where;

L_0=Length of muscle at P_0

L_1=Length muscle at P_1

P_0=Initial tension at the start of linear phase

P_1=Final tension at the end of linear phase.

This equation (2) calculates the coefficient of linear shortening with respect to the initial length but since the muscular activity was recorded at resting length, therefore, instead of initial length, the resting length was used in the formula.

$$K = \frac{L_1 - L_0}{R.L.(P_1 - P_0)} \qquad (3)$$

Where;

R.L.=Resting length

This equation (3) was used for the calculation of the coefficient of linear shortening for all the muscles used in the present study.

All the calculations, statistical analysis and the graphs were made by using computer facility with Lotus release 3 and for comparison purposes student's t-test was used.

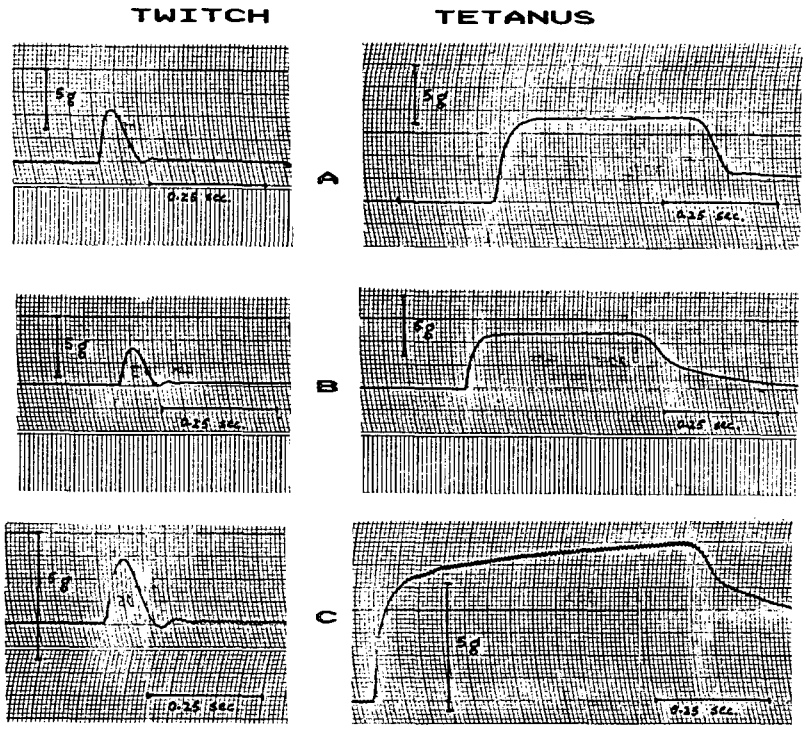


Fig. 3. Auxotonic twitch and tetanus records obtained from sartorius, gastrocnemius and rectus abdominis muscles. A: SARTORIUS, B: GASTROCNEMIUS, C: RECTUS ABDOMINIS

RESULTS

All the twitch and tetanus records obtained from sartorius, gastrocnemius and rectus abdomins muscles were used for the measurement of various auxotonic parameters. Typical records obtained from these muscles are shown in Fig. 3.

Maximum shortening

Our results regarding the maximum shortening (S_{max}) in twitch and tetanus showed significantly ($P<0.005$ & $P<0.0005$) higher values in the SAR muscles when compared with GAS ones. When this comparison was made between SAR and RAB muscles significantly ($P<0.01$) higher values were observed only for tetanic shortening in the RAB muscles. In addition, a statistical comparison of S_{max} has demonstrated significantly higher values of maximum shortening for both the twitch ($P<0.01$) and tetanus ($P<0.005$) in RAB muscles than those of GAS ones (Table 1). Moreover, when S_{max} was compared between twitch and tetanus, significantly ($P<0.0005$) higher values were observed in tetanus for all the three experimental muscles.

Auxotonic tensions

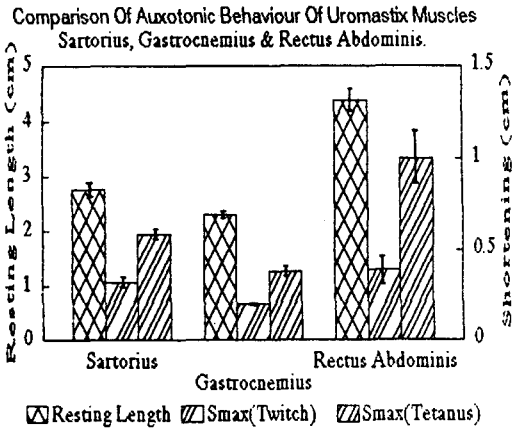
Auxotonic tensions produced by twitch and tetanic stimulations have demonstrated significantly ($P<0.0005$) higher values in SAR muscles than those of GAS ones. However, when this parameter was compared between SAR and RAB muscles significantly ($P<0.005$) higher values were observed only for twitch tensions in the SAR muscles. On the contrary when auxotonic tensions were compared between GAS and RAB muscles higher values were observed for both twitch and tetanus in the RAB muscles being significant ($P<0.005$) for tetanus only. On the other hand a statistical comparison of this parameter between twitch and tetanus for all the three experimental muscles demonstrated

Table 1. Mean values of various parameters of auxotonic twitch and tetanic contractions, recorded from the sartorius, gastrocnemius and rectus muscles of reptile, Uromastix hardwickii. These muscles were stimulated with 50V pulses of 5ms duration. For tetanus recordings. the frequency of stimulation was 80/sec.

S. No.	Parameters	Sartorius Meam+S.E.	P*	Gastrocnemius Mean+S.E.	P**	Rectus abdominis Mean+S.E.	p***
1.	SHORTENING (cms.)						
	Twich	0.319±0.029(6)	P<0.005	0.197±0.007(6)	P<0.01	0.383±0.061(6)	P>0.05
	Tetanus	0.587±0.029(6)	P<0.0005	0.378±0.026(6)	P<0.005	1.000±0.141(6)	P<0.01
	Twitch Vs Tetanus	P<0/0005		P<0.0005		P<0.0005	
2.	TENSIONS (Kg/cm^2)						
	Twitch	0.016±0.001(6)	P<0.0005	0.009±0.001(6)	P>0.05	0.011±0.001(6)	P<0.005
	Tetanus	0.029±0.002(6)	P<0.0005	0.016±0.001(6)	P<0.0005	0.032±0.003(6)	P>0.05
	Twitch vs Tetanus	P<0.0005		P<0.0005		P<0.0005	
3.	COEFFICIENT OF LINEAR SHORTENING ( ):						
	Twitch	-0.27±0.002(6)	P>0.05	-0.03±0.001(6)	P<0.01	-0.044±0.005(6)	P<0.005
	Tetanus	-0.029±0.002(6)	P>0.05	-0/033±0.001(6)	P<0.05	-0.039±0.003(6)	P<0.01
	Twitch Vs Tetanus	P>0.05		P<0.05		P>0.05	

The figures in the parenthesis represents the number of muscles used.

P*, P**, and P*** represents the significance levels between SAR & GAS, GAS & RAB and SAR & RAB, respectively.



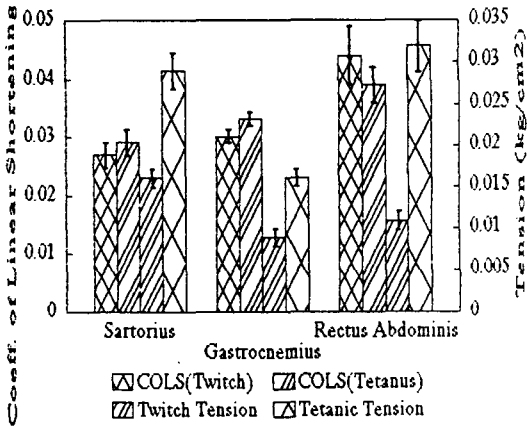


Fig. 4. Comparison of auxotonic behaviour of uromastix muscles sartorius, gastrocnemius & rectus abdominis.

significant (P<0.0005) differences, being higher in the tetanus (Fig. 4).

Coefficient of linear shortening

A statistical comparison of coefficient of linear shortening (COLS) between SAR and GAS muscles for both twitch and tetanus has demonstrated insignificant differences among them. However, a similar comparison between SAR and RAB muscles has demonstrated significantly different values for muscles. Similarly, significantly higher values of this parameter were also observed for both twitch and

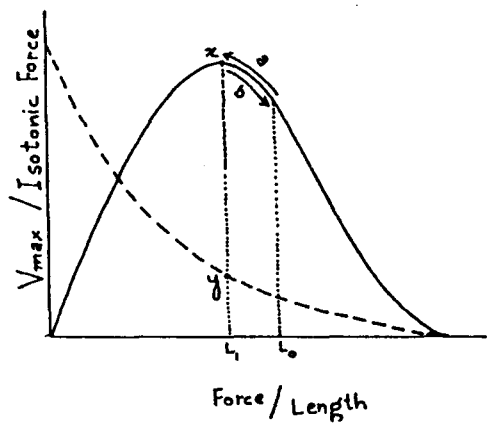


Fig. 5. Hypothetical diagram showing superimposed PV and LT relations. Dotted vertical lines represents the range of auxotonic activity of our experimental muscles. At the time of stimulation, muscle was at length 'Lo' which then reduced to L_1 (represented by arrow a) during contraction i.e., shortening and then comes back to Lo (arrow b) during relaxation i.e., lengthening. According to the diagram, this activity is happening at the peak of the active tension curve in its descending portion. The points x and y in LT (full line) and PV (dashed line) curves respectively represents the auxotonic activity of experimental muscle (for maximum tension and maximum speed of shortening at resting length), representing maximum efficiency (Wilkie, 1968; Fig 4-7b).

tetanus in the RAB muscles being, P<0.01 and P <0.05, respectively to those of the GAS ones. However, comparison of COLS between twitch and tetanus demonstrated higher values in tetanus. However, this difference was significant (P<0.05) in GAS while, insignificant in SAR muscles. On the other hand, it was higher in twitch in RAB muscles those of their tetani, being statistically insignificant (Fig. 4).

DISCUSSION

Justification and validity of the method used for the determination of Shortening (S_{max}) and Coefficient of Linear Shortening (COLS)

It is a common fact that the displacement of

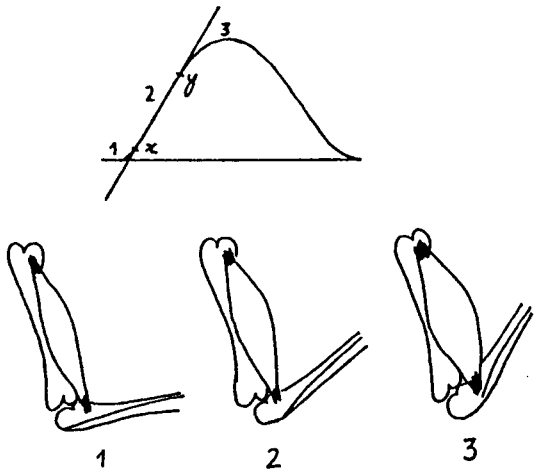


Fig. 6. Hypothetical diagram of twitch: The line xy represents phase of linear shortening. Phases 1, 2 & 3 have been correlated with invivo muscle activity resulting in limb movement under a given load. Phase 2 is the region in twitch that represents linear shortening in muscle resulting in linear movement of the limb. The phase 3 represents the region in which the internal viscosity elasticity is involved.

the calibrated transducer can only be used for tension measurements and it cannot provide a mean for the measurement of shortening produced. In the present work the method used for the measurements of shortening is found useful and accurate. According to this procedure, the displacement of the transducer was converted by a formula into exact magnitude of change in muscle length that occurred due to shortening (Materials & Methods).

Bahler et al. (1967) have reported that the velocity of shortening achieved at a given length was lower for longer initial lengths. In the present experimental work the initial length of muscle was the resting length at which maximum extent of shortening (S_{max}) as well as auxotonic tensions were found to be different for different muscles of Uromastix. In addition, the change in muscle length and tensions, measured at the steepest rising phase of the tension records (during which rapid change in length i.e. shortening and tension occurs) was used for the calculation of COLS. It is to be pointed out here that during the whole contraction phase the muscle cannot produce a linear change in its length due to the presence of non-uniform as well as non-homogenous structural elements in it. It is therefore the reason that greater the non-homogeniety, greater the curved response is observed in terms of the shape of twitch. However, during this contraction period there is a phase that represents linear response (See Fig. 2 in materials and methods). This linear phase was therefore used for the measurement of COLS and it was considered that during the steepest rising phase of the records the speed of shortening (determined as COLS) would be maximum in terms of efficiency (Wilkie, 1968), because the muscle was at resting length. It is to be pointed out that we have applied a physical principle to derive the equation of coefficient of linear shortening that expresses the expansion of solids with temperature, since the physical state of skeletal muscle is also temperature dependent and further their behaviour obeys the thermoelastic principles (Hill, 1952). Moreover, the application of coefficient of thermal expansion was also considered earlier in muscle by Hill (1970) regarding elastic energy and thermoelastic heat.

In fact, the concept of the measurement of COLS was to find out a criterium to differentiate the structural inhomogenieties of different muscles on the basis of the ability of linear shortening in them. Moreover, the linear phase of twitch at which slope was drawn for the determination of L_0, L_1 and P_0, P_1 may be related with the active tension and force-velocity curves as explained in Fig. 5. The applicability of L-T and P-V, curves on whole muscle as well as on single muscle fibre (Muhl, 1982) justifies the measurement of COLS from whole muscles to single fibres in similar manner.

Further, we are of the opinion that the movements of limb in vivo against a load also involve similar kind of linearity (rapid movement) and non linearity (slow movement) with the participation of only contractile and both the

contractile and elastic elements (internal viscosity), respectively. This concept is explained in Fig. 6.

Our experimental results regarding the maximum auxotonic shortening and tension produced by sartorius (SAR), gastrocnemius (GAS) and rectus abdominis (RAB) muscles have demonstrated greater magnitude of shortening in the muscle that possess greater resting length (Table 1). According to these results RAB muscles of Uromastix have the ability to produce greater shortening and tension than those of SAR and GAS muscles. It is to be pointed out here that muscle fasciculi/fibre arrangement in a muscle exercise prominent effect on muscle mechanics. In this connection, it is expected that larger the muscle used greater will be the shortening produced, provided that it possess greater number of contractile units and elongated strap like fibre arrangement (Gray's Anatomy). In view of this expectation, out of the three muscles used in present experiments, the SAR muscle has the expected architecture being the best strap like arrangement while, the RAB also fall into this category of strap like muscle but it possess tendinous intersections as well. This comparison of the architecture between SAR and RAB muscle didnot fit exactly on our experimental results regarding shortening and tension generation by the two muscles. However, this deviation may be explained in terms of size of the myofilaments, contractile units and cross bridges in these muscles. Simmons and Jewell (1974) have suggested that muscles with longer sarcomeres and filaments should produce more tension per unit cross-sectional area because there are more cross-bridges in parallel in the basic unit. Further Zachar and Zacharova (1966) have compared the ratio of A-band length and tension between crayfish and frog muscle fibres and concluded that greater the length of A-band, the greater will be tension produced. In the present work histological studies were not carried out. However, on the basis of supporting arguments like measurement of tension, maximum shortening velocity and actomyosin ATPase activity in different type of muscles (Simmons and Jewell, 1974) may be used for the existence of bridges and their cyclic activity. Therefore, on the basis of greater tensions and shortenings observed in RAB than those of its SAR and GAS muscles, elongated myosin filaments i.e., length of A-band in sarcomere of RAB muscles are proposed (Zachar and Zacharova, 1966).

The analysis of auxotonic behaviour of GAS has demonstrated that this muscle exhibits lesser tension and shortening magnitudes as its characteristics. Azeem and Shaikh (1987) have demonstrated the architecture of GAS muscles of this animal possessing straight as well as oblique fibre arrangement in the two of its bellies, being short and unipinnate as well. It was therefore the reason that this muscle developed lesser magnitude of shortening as well as tension. Further presence of greater elastic elements in this muscle may also be responsible to increase the internal viscosity that had resisted the act of shortening.

In the present experiments the speed of shortening of the three experimental muscles was also determined in terms of COLS. These results demonstrated greater speed of shortening in RAB and intermediate in GAS while, lowest in the SAR muscles. These differences in the speed of shortening demonstrates that greater magnitude of auxotonic tension and shortening are associated with greater change in length ($\triangle L$) during the steepest rising phase of twitch and tetanus and hence represents greater speed of shortening (Langfeld et al, 1989). However, RAB is considered as one of the slow muscles of the body and shortening as well as tension generation ability of slow and fast muscles is different. Further speed of shortening is usually reported to be lesser in the slow muscles. In this connection, occurrence of Type 1 fibres in coleius muscles of rat being greater than 70% while of Type 2 fibres in EDL and PL muscles being greater than 90% have been reported by Ranatunga and Thomas (1990). In the RAB muscles of Uromastix the observed speed of shortening suggests that the fibre population is probably different in this muscle to other slow muscles and occurrence of greater number of

Type 2 fibres in this muscle cannot be ignored. However, one more explanation may also be given for the faster speed of shortening in this muscle. Simmons and Jewell (1974) have suggested that at a given fibre length having shorter sarcomeres being more in series have faster rate of shortening. It is therefore concluded that probably a greater length of A-band is responsible in RAB muscle for greater tensions and shortenings while, due to tendinous intersections, greater number of shorter sarcomeres in series were probably responsible for faster speed in this muscle.

According to our discussion it is clear that our results were according to the expectations in terms of muscle architecture and therefore the measurement of shortening as well as the determination of speed were correct and on the basis of COLS the muscle inhomogenities may be determined in terms of contractile and elastic elements. However, the differences in the mechanics of RAB muscles of this animal to those of other slow muscles in mammals suggests that either it possess different fibre types or it may have different muscle fibre/A-band/sarcomere length (Mackay and Harrop, 1969; Zachar and Zacharova, 1966; Azeem, 1992).

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