In Figure A) typical anastomosing fluorescent nerve fibres with their intensely fluorescent varicosities can be seen in a control atrioventricular valve.

A marked decrease in the specific fluorescence of the valvular nerve plexuses could be demonstrated following the i.v. administration of scorpion venom to the rats. The degree of reduction was dependent on the interval between administration and death. A general reduction was seen within 30 min, and after 60–120 min there were almost no nerves clearly visible in about 70% of cases. Only a few weakly fluorescent fibres remained visible. The decrease in fluorescence was located mainly to the varicosities.

Figure B) shows very weakly fluorescent nerve fibres and barely visible varicosities in an atrioventricular valve from a rat which had been injected with scorpion venom 60 min before death.

Discussion. The results of the present investigation clearly demonstrate that administration of Brazilian scorpion venom (*Tityus serrulatus*) to adult rats causes a depletion of catecholamines in the sympathetic nerves from the atrioventricular valves. Some of the symptoms of scorpionism in vertebrates are ascribed to a sympathomimetic overactivity. Elevation of plasma adrenaline content and of catecholamines and their metabolites in the urine in patients after scorpion sting has been reported[19,20]. More recently it was shown that scorpion venom produces an increase in blood pressure accompanied by a massive discharge of catecholamines into the blood[21]. The cardiac stimulation which follows administration of scorpion venom has been shown to be a peripheral phenomenon, probable through the release of tissue catecholamines[5]. These and other manifestations of scorpion envenomation, such as myocardial lesions[22], pulmonary edema[16], tachycardia[5], piloerection[23], and mydriasis[23], could be due to the release of noradrenaline and other amines from the adrenergic nerve endings. Scorpion venoms markedly influence excitable membranes, altering the permeability to various ions with resulting electrophysiological and physicochemical changes[24,25]. Probably scorpion venom releases catecholamines by activating the fundamental physiological mechanism.

Further experiments, to investigate the neurotransmitter-depleting action of Brazilian scorpion venom on sympathetic nerve endings, are in progress.

Zusammenfassung. Mittels Fluoreszenztechnik wurde ein mikrochemischer Nachweis der Freisetzung von Katecholamin an adrenergischen Strukturen in den Arterioventrikularklappen des Rattenherzens nach Verabreichung von Skorpiongift (*Tityus serrulatus*) erbracht.

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The Effect of Applied Tension on the Length of Striped Muscle

The effect of applied tension on skeletal muscle has considerable relevance to orthopaedic surgery. Forceful stretching of contracted muscles forms an important part of treatment in many patients with musculo-skeletal deformities. In addition, limb traction is frequently used to maintain the position of a fractured bone after reduction, or to overcome painful muscle spasm.

Although many workers have described the relationship between tension and length in isolated muscle fibres, or in actively contracting muscles, much less has been written on the effects of applied tension on individual muscles. In view of the importance of this aspect of muscle physiology in orthopaedic practice, a study of the effect of applied tension on the length of intact skeletal muscles was undertaken.

Materials and methods. 30 male Wistar albino rats weighing 285–400 g were used to assess the effect on muscle length of increasing, followed by decreasing tensions. The animals were divided into 3 groups of 10. Each group was used to study 1 of 3 selected muscles, namely the tibialis anterior, the tibialis posterior and the peronei taken as a single entity. The tendons of the 4 rat peroneal muscles together provide a tendinous cord above the ankle joint, just large enough to be handled.

An apparatus was constructed which allowed known tensions to be applied to the living muscles and permitted their length to be measured. It consisted of a wooden base, at one end of which a heavy wooden block was screwed down. The anaesthetised rat was laid upon this block and was prevented from sliding off it during application of tension by two small posts. The rat's tail lay between the posts and its hind limbs on either side.

A length of No. 36 gauge stainless steel wire was tied firmly to the distal part of the tendon of the muscle under investigation. The other end of the wire was fixed to the cantilever incorporated in a Pye TS1 strain gauge. This was rigidly mounted on a movable brass block whose movement was controlled by turning a finely threaded screw. The strain gauge and movable brass block were fixed to the wooden base of the apparatus opposite the anaesthetized rat (Figure 1). By moving the strain gauge in the longitudinal axis of the rat's hind limb, the tension in the muscle under investigation was varied as required.

The tension applied to a muscle was measured electrically by incorporating the strain gauge as one of the resistances of a Wheatstone Bridge. By applying tension to the strain gauge its resistance was altered. A galvanometer connected across the two ends of the bridge indicated any alterations in the balance of the circuit. The galvanometer was calibrated before each experiment by attaching known weights to the strain gauge. The range of tensions used varied from 0–200 g.

Measurements of muscle length under different tensions were made with a travelling microscope placed between the rat and the strain gauge (Figure 1). The rats were anaesthetized by an i.p. injection of 6–12 mg Nembutal,

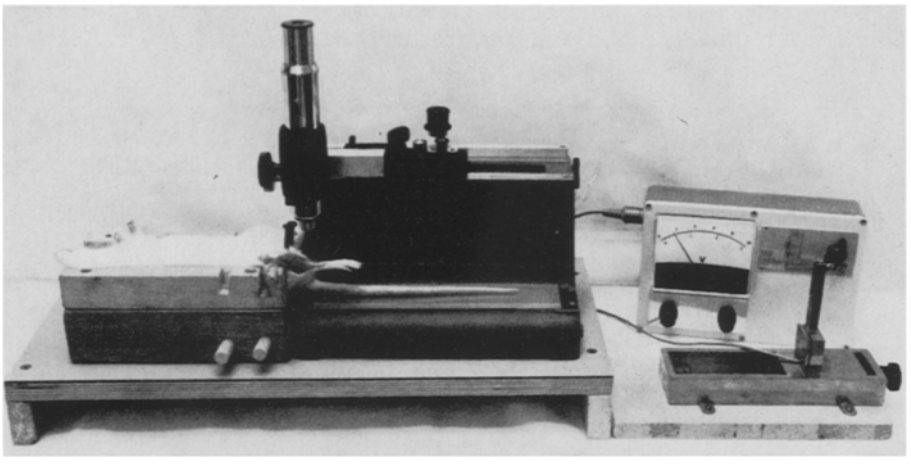

Fig. 1. A general view of the apparatus. The living rat is supported by a wooden block and the tibialis anterior muscle is exposed. A steel wire is attached to the tendon and connected to the strain gauge in front of the galvanometer. In the middle is the travelling microscope (see text).

supplemented by open ether. The skin overlying the selected muscle was removed and its tendon severed from its distal attachment. The exposed tissues were kept moist with saline. The stainless steel wire was tied firmly to the tendon and was then connected to the strain gauge. Fixed points for measurement were provided by inserting a fine needle into the tibia at the most proximal point of attachment of the muscle and by making a small mark with methylene blue upon the tendon distally. The distance from one edge of the needle to the upper limit of the blue mark was measured using the travelling microscope which was fitted with a Vernier attachment. In this way, serial length measurements at applied tensions varying from 0–200 g were made, both as the tension was increased and then reduced. At the completion of the experiment, the rat was killed by an overdose of ether.

Results. Cartesian graphs were first plotted, showing the variation of the mean percent change in muscle length with tension. For example, Figure 2 shows the graph for the peronei. The graphs for the 3 muscles investigated suggested that a maximum increase in length occurs on the application of tension. The maximal elongation obtained during stretch tended to be maintained at lower tensions during relaxation (Figure 2).

When the logarithm of the mean percent change in muscle length was plotted against applied tension (Figures 3, 4 and 5), a complex exponential relationship between the two was suggested. In particular, the lie of the mean data points suggested that 3 distinct phases occurred when the muscle tension was varied. These were recognizable as abrupt changes in the slope of the semi-log plots of the data. They occurred when the muscle was stretched and relaxed. Judged by naked eye observation, phase 1 (stretching from zero to tension) had a comparatively steep slope. This was succeeded by a less steep phase 2, which was followed by a terminal horizontal phase 3. Although the phase pattern was the same on stretching or relaxing the muscle, each phase appeared to be spatially distinct.

This was confirmed by regression analysis of the stretch and relaxation data obtained for the tibialis anterior (Table) and also for the tibialis posterior and the peronei. It is emphasized that the tension range for each phase in the Table was selected by observation of the semi-log plots of the raw data. The slope of phase 1 was considerably greater than that of the intermediate phase 2. Both slopes were significantly different from zero.

Phase 3 whether on stretch or relaxation was represented by a horizontal line, in that its slope was not significantly different from zero. The relaxation phases were not coincident with the stretch phases and on release of tension the muscle did not return to its initial resting-

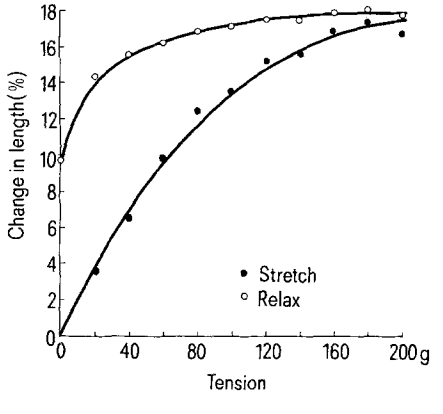

Fig. 2. Cartesian graph showing the change in muscle length on stretching and then relaxing the peroneal group of muscles. Each point is the mean of 10 readings.

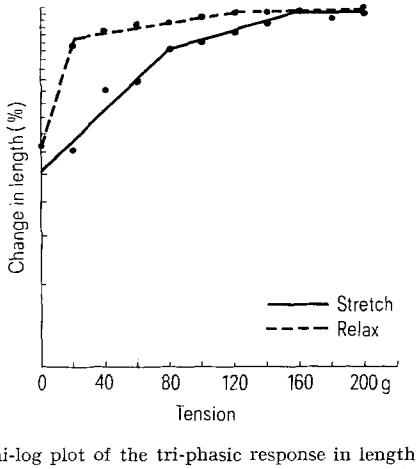

Fig. 3. Semi-log plot of the tri-phasic response in length of tibialis anterior to applied tension. The muscle was observed in situ with intact nerve and vascular supply. Each point represents the mean of ten readings from 10 different animals.

Table showing the range of tensions in the 3 phases of stretching and then relaxing the tibialis anterior

Phase	Tension (g)	Slope	S.E. Slope	Intercept	$P <$
Stretch					
1	0–80	0.0184	0.0048	1.2301	0.001
2	80–160	0.0043	0.0015	2.2461	0.01
3	160–200	0.0013	0.0011	2.7116	Not significant
Relax					
3	200–120	0.0003	0.0010	2.8903	Not significant
2	120–20	0.0053	0.0018	2.4123	0.01
1	20–0	0.0521	0.0116	1.6343	0.001

In phase 3 it is noted that no significant change in length of the muscle occurs during stretch or relaxation.

length (Figures 3, 4 and 5). The maximum increase in length on application of tension was 18.9% ± 1.3 of the resting length in the case of the tibialis anterior; 18.0% ± 1.28 in the case of the peronei; and 13.9% ± 1.30 in the case of tibialis posterior.

Discussion. The results show that the muscles investigated, namely the tibialis anterior, the tibialis posterior and the peroneal group, responded to tension by a limited increase in length which was not greater than 20% of the length of the resting muscle. Furthermore, the relationship between change in muscle length and applied tension was a complex exponential. In all 3 muscles the response was tri-phasic, the third terminal phase showing a negligible change in length however great the applied tension.

Considerable emphasis has rightly been placed on the molecular structure of myofibrillae in the mechanism of contraction in living muscle. The present investigation confirms the observations of WILKIE[1] that when a muscle is subjected to applied mechanical tension the ensuing elongation is exponential in type. However, the tri-phasic nature of the response does not appear to have been commented upon previously.

The mechanism controlling the tri-phasic response of a muscle to stretch is uncertain and certainly complex. The two successive responses (phases 1 and 2) of a muscle to applied tension observed here are probably related not only to the disposition of actin and myosin filaments in the myofibrillae, but also to the connective tissue and the fascicular architecture of the whole muscle.

The connective tissue of muscle shows at least 2 distinct regions, the endomysial partitions around each muscle fibre and larger, interfascicular or perimysial fibrous aggregations. NAGEL[2] described fine collagenous fibrils which wind along the individual muscle fibres in a helical fashion and make frequent connection with longitudinally orientated collagen bundles. These latter coarser bundles comprise the outer layer of the endomysial sheath. When the muscle is stretched, the spiral fibres lie longitudinally and parallel to the outer endomysial fibres. NAGEL[2] considered that in this way the muscle was protected from excessive strain.

The perimysial connective tissue consists of considerably thicker partitions than the endomysial sheath. These are interfascicular and contain many elastic fibres as well as collagenous and fatty material. FENEIS[3] suggested that movement of one fasciculus on another was facilitated by the perimysial elastin.

From a consideration of these factors concerning the connective tissue elements of striated muscle and of what is known of the structure of the myofibrillae, it is possible to suggest an explanation of the tri-phasic response of muscle to applied tension. In phase 1 (stretch) sliding of the muscle fasciculi upon each other occurs, this movement being assisted by the presence of elastic fibres within the

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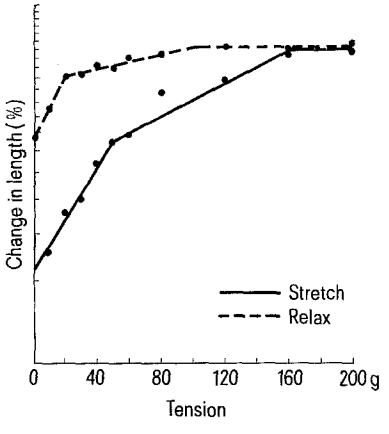

Fig. 4. Semi-log plot of tri-phasic response in length of tibialis posterior to applied tension.

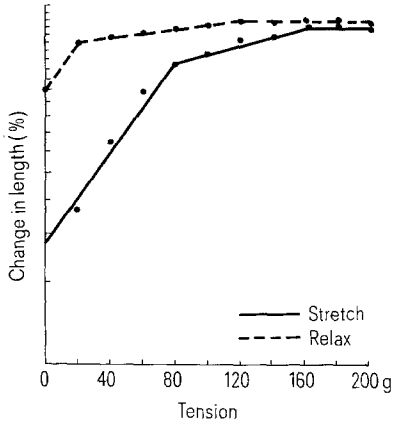

Fig. 5. Semi-log plot, as in Figures 3 and 4, of the peroneal group of muscles.

perimysium. In addition, it may be expected that the area of overlap between the actin and myosin filaments within the myofibrils is decreased, leading to a widening of the H- and I-bands. But the tension generated within the myofibrillae and opposing the stretching force, is potentiated by the activity of the muscle spindles. COOPER and DANIEL[4] have shown that these are active at all lengths and tensions of muscle.

In phase 2 stretch, the actin and myosin filaments continue to separate, but the rate of increase in length is reduced although the applied tension continues to rise. It may well be that the fine helical collagenous fibres of the endomysium surrounding the muscle fibrils are stretched in phase 2 until they lie parallel to the myofibrillae. These collagenous fibres are not elastic and their resistance to a stretching force is greater than that of the elastic fibres in the perimysium. This may account for the reduced rate of lengthening which occurs in phase 2.

In phase 3 (stretch) the myofibrils are maximally stretched and no further elongation can occur without their disruption. The helical fibres of the endomysium are also fully extended and protect the myofibrils by their resistance to stretch. The result is that no further increase in the length of the muscle occurs although the applied tension may continue to rise.

When tension is relaxed, phases 2 and 3 are prolonged and the muscle is still longer at the end of phase 1 than it was at the beginning of the experiment. Probably a slow re-arrangement of muscle collagen occurs from a stretched parallel pattern to the unstretched helical form. This prevents the muscle from regaining its normal length immediately applied tension is relaxed. The inelastic collagen bundles require active muscle contraction in order to restore their arrangement to that present in the resting muscle before it was stretched.

A large part of the literature on the physiology of muscle is devoted to the maximum tension generated on nervous stimulation. In contrast, it is emphasized here that a muscle does not behave like an elastic band when it is stretched, a matter of considerable orthopaedic importance. In orthopaedic practice tension is applied to the limb as a whole when attempts are made to stretch muscles. Because the maximum possible increase in muscle length, at the most 20%, has been achieved by the end of phase 2, further increase in tension can have no effect on the muscles as far as increasing their length is concerned. Other structures in the limb such as the nerves and joints as well as the muscles themselves may be injured when subjected to forceful stretching. The tendency to increase the force applied to the limb in order to achieve greater lengthening of muscle should therefore be resisted. Not only can it but fail to lengthen muscle further; it may be positively harmful.

Zusammenfassung. Nachweis, dass sich der Skeletmuskel bei Streckung dreiphasisch verhält, wobei sich in der letzten Phase seine Länge auch bei grösserem Zug nicht mehr ändert.

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Water Movement During Diuresis in the Tsetse Fly (*Glossina austeni*)

Tsetse flies take blood meals at least equal to their own weight at regular intervals[1]. This imposes severe limitations on the flying ability of the fly[2], and it is therefore advantageous that the fly should eliminate the water from the blood meal in the shortest possible time. In fact, *Glossina* often begins diuresis in the first minute following the commencement of feeding and it has been shown that in male *Glossina*, 38% of the total weight of the blood meal is excreted during the 30 min after feeding[3]. Diuresis in female *G. austeni* is even more rapid with more than 40% of the total meal weight being excreted in the first half hour after feeding (unpublished observations). The excretion of water is more rapid than that reported for other haematophagous insects and the speed with which the first drops of urine appear led me to investigate the problem of water movement during diuresis in female *G. austeni*. Did water from the blood meal in fact pass into the haemolymph to be coincidently removed by the Malpighian tubules, under the influence of diuretic hormone?

LESTER and LLOYD[4] were the first authors to describe the role of the Malpighian tubules in excretion in tsetses and based their results on observations of water movement within the tubules during diuresis. Prior to this, however, NEWSTEAD, DUTTON and TODD[5] had hypothesized that the rapid excretion of water in *Glossina* resulted from the clotting of the blood meal in the midgut with the subsequent passage of serum directly down the gut. LESTER and LLOYD[4] discounted this hypothesis by feeding flies on both haemolyzed blood and on blood containing methylene blue and by observing that neither the haemoglobin nor the dye appeared in the urine. However, their experimental results are not convincing because of the unphysiological conditions used for feeding and therefore, I have observed the excretion of both ^3H_2O and a large, presumably unmetabolized molecule, ^{14}C-dextran, following feeding.

Materials and methods. Female *G. austeni*, kindly supplied by Dr. P.A. LANGLEY, during their second reproductive cycle, were fed on fresh defibrinated bovine blood after the method of LANGLEY[6] and MEWS (unpublished), containing 125,000–145,000 dpm ^3H_2O (NEN, specific activity 1 μCi/μl) per μl and 1500 dpm ^{14}C-carboxyl dextran (NEN, specific activity 781 mg/mCi, M.W. 60,000–90,000) per μl. The flies had been previously reared on goats and were fed on the previous day, in one instance, and 2 days previous in another instance. Samples of urine were taken after 5 min, 15 min and 30 min from the cessation of feeding and placed directly into scintillation vials containing 10 ml of 1% butyl-PBD, 10% ethanol and 50 m*M* acetic acid in toluene. Samples were counted in a Packard Tricarb Liquid Scintillation Spectrometer using doublelabel settings and both quench correction and spillover correction were performed using external standardization. In some instances, samples of haemolymph were also obtained from

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