ficantly different from that for 0 pg of testosterone. The coefficient of variation of each standard curve point assayed in triplicate was always less than 7%.

Specificity of this method was evaluated by investigation of blanks, antibody specificity, and comparison of results obtained with column chromatography with those obtained without. The specifity of our antiserum was tested by direct incubation with 15 other steroids. Percent cross reaction was calculated according to the method of ABRAHAM[8]. Table I shows the cross reactivity of our antibody to different steroids. Recovery of labelled testerone without column chromatography was improved. Pre-extraction with hexane was still a necessary step.

Table II. Pooled plasma testosterone values obtained after radioimmunoassay with and without column chromatography (mean $\pm$ S.D. ng/100 ml)

	Column used	Column omitted
Male	$538 \pm 59\ N = 56$	$528 \pm 30\ N = 40$
Female	$40 \pm 8\ N = 35$	$41 \pm 7\ N = 9$

Normal testosterone values for male and female are shown in Table II. These results agree with other methods. Thus, samples may be assayed without the use of column chromatography; this column omission procedure is simple, accurate, sensitive, specific, and suitable for clinical use.

Résumé. On a développé und méthode simple pour déteminer sans chromatographie en colonne le testostérone du plasma. La purification a été faite par préextraction avec l'hexane. Un antisérum contre le testostérone a été produit chez des lapins inoculés avec des conjugués de testostérone-3-oxine et d'albumine de sérum bovin.

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Simultaneous Measurement of Velocities of Adjacent Sacromere Length Changes in Single Muscle Fibres

Light, phase contrast, interference and polarizing microscopy have been applied to the study of structures of living muscle fibres. To measure dynamic changes during muscle contraction and relaxation, various transducers have been used to record tension; whereas changes in striation pattern of muscle fibres are recorded either by photoelectric devices with markers applied onto the muscle fibres, or by means of high-speed cinematography[1-4]. Recently changes in sarcomere length during isometric contraction of isolated frog muscle fibres were studied using laser diffraction techniques. The diffracted light was then projected on a screen for photography[5]. The marker techniques however do not indicate accurately the individual sarcomere lengths, and analysis of cinematography has the disadvantage of being tedious and time consuming. A method for measuring the different velocities of sarcomere length changes during contraction and relaxation of single striated muscle fibres has been developed.

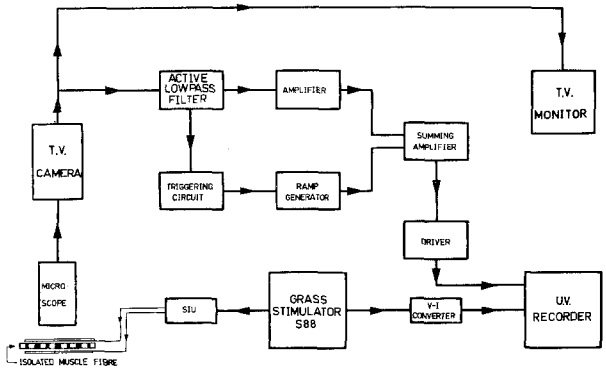

Fig. 1. Block diagram for measurement of sarcomere length and velocity of contraction in single striated muscle fibre. SIU, stimulus isolation unit.

Materials. The accessory muscle located in the coxatrochanteric joint of the Asiatic horseshoe crab, *Tachypleus gigas* is used. This muscle is essentially a receptor muscle with sensory neurons attached to it for the detection of joint movement[6]. The broad striations (2–12 μm) and small diameters (2–6 μm) of these fibres are very favourable for signal identification and separation. Morever, electron microscopic studies have revealed this muscle to be devoid of H zones[7,8].

Method. Isolated single muscle fibres are fixed at one end by a stainless steel spring, clipped onto the exoskeleton and bathed by oxygenated filtered sea water in a lucite chamber. The tendon end of the muscle fibres is attached by means of a stainless steel microhook to a special micromanipulator constructed in our laboratory. The isolated single fibres could then be stretched by the micromanipulator to varying degrees of the resting length. The muscle fibre is then observed under a phase contrast microscope (Olympus, Vanox NH $40\times/0.65$ or NH $100\times/1.30$). The image is also displayed through a closed circuit T.V. system (ITC. CTC-5000 T.V. camera) on a monitor (Pye Model 59). An adjustable slit is placed on top of the photo eye-piece (Olympus P-15$\times$) to limit the

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field of observation. Striations of the muscle fibre are then aligned vertically on the T.V. monitor so that the necessary information on sarcomere lengths is contained within one T.V. frame (Figure 1). The video signal which varies with the optical density of the fibre striations is first passed through a third order Butterworth lowpass filter using a current realized negative impedance converter (INIC) with a −3db frequency at 1.5 kHz. The low frequency signal is amplified, so that the A bands and Z lines appear as upward deflections on an oscilloscope (Tektronix 502A). The A bands are represented by large and wide peaks, and the Z lines by peaks of smaller amplitudes. The distance between the adjacent Z line peaks is then the sarcomere length.

To measure velocity, the low frequency signal from the sarcomeres is superimposed on a 50 Hz ramp signal via a summing amplifier (Philbrick Model 1009 operational amplifier), before being fed to a UV-recorder (SE Laboratory Model 2006). The ramp signal itself is generated by a three mode integrator (Philbrick Model 4850) which is reset by a 50 Hz triggering pulse from the T.V. frame pulse. The mixed signals then appear as distinct deflections riding on the ramps and when the UV-recorder is operated at a speed of 10 mm/sec, the ramps are closely packed together. With the muscle fibre at rest, the relative distances between the A bands and the Z lines are constant, the deflections appear as horizontal dark strips on the UV-records. Any change in sarcomere lengths will be indicated by a corresponding change in the distances of separation between the dark strips. The slopes of the dark strips then indicate the velocities and changes of slopes, acceleration or deceleration (Figure 2).

To stimulate the muscle fibres electrically, fine silver wires are placed one on each of the isolated single fibres. Rectangular pulses are provided by a stimulator (Grass S88) through isolation circuits. The same pulses are fed to a current converter for driving an event marking galvanometer in the UV-recorder.

Result. Figure 2 (A) shows a total of 4 Z lines and 3 A bands i.e. 3 complete sarcomeres. During electrical stimulation, due to the series elastic components in the system, there is a net upward movement of the fibre, i.e. movement towards the exoskeleton end, even though the fibre is fixed at both ends. Only movements of the lower 3 Z lines and 2 A bands could be followed throughout the course of contraction. The record clearly indicates that sarcomeres could shorten to different extent even under the same degree of tension. Thus at the height of contraction sarcomere 1 shortens from 6.6 μm to 5.6 μm i.e. 15%; whereas sarcomere 2 shortens from 8.1 μm to 5.7 μm i.e. 30% (Figure 2B). Figure 2C shows the tracing of the Z lines. The adjacent Z lines (Z_1, Z_2, Z_3) are observed to move at different velocities.

Discussion. Our technique therefore provides a convenient method of studying sarcomere lengths as well as different velocities of contractions of sections of isolated single fibres, viz. Z lines and A bands. Ordinarily the use of light microscopy is inadequate to resolve the A bands and Z lines of isolated muscle fibres due to the thickness of the fibre compared with the length of the sarcomeres[9]. The accessory muscle of the Asiatic horseshoe crab overcomes this difficulty by possessing very broad striations and very small diameters. Studies have now been initiated to investigate the length-tension relationship of such muscle fibres using the technique described above.

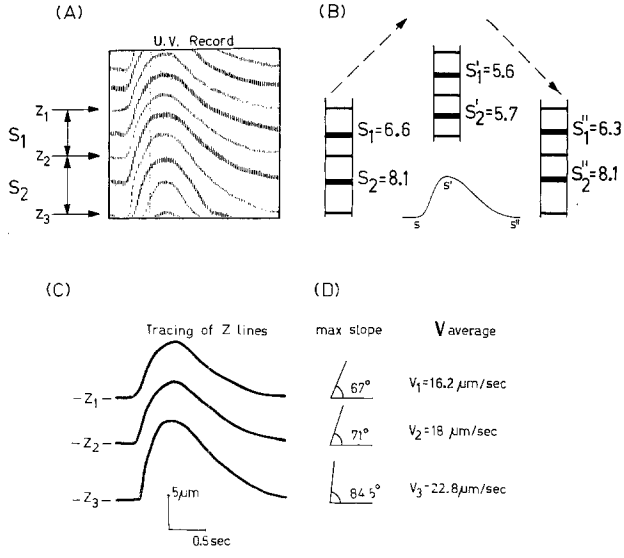

Fig. 2. A) Actual UV record of single contraction of a portion of muscle fibre in response to single shock stimulation obtained with the system described in Figure 1. Z, Z-lines of isolated living single muscle fibre; S_1, sarcomere 1; S_2, sarcomere 2. B) Diagramatic representation of the portion of muscle fibre in the vidicon field. S, sarcomer length in μm; S, initial state; S′, contracted state; S″, relaxed state. C) Tracing of Z lines. D) Maximal slopes and average velocities of Z lines displacements.

Zusammenfassung. Messmethode für die Sarkomerenlänge der gestreiften Einzelmuskelfaser. Bei elektrischer Reizung verkürzen sich benachbarte Sarkomere ja nach Ausgangslänge und benachbarte Z-Linien bewegen sich mit verschiedenen Geschwindigkeiten selbst unter «isometrischen» Bedingungen.

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Acetylcholine-Induced Atrial Tachyarrhythmia: A Modification to Quantitate Potency and Duration

Acetylcholine-induced atrial fibrillation has been used extensively as a method for evaluating potential anti-arrhythmic agents[1]. As with most anti-arrhythmic methods, this particular technique suffered from the lack of adequate controls. Frequently, it was difficult to determine whether reversion of the arrhythmia was drug induced or occurred spontaneously. Schallek[2] improved on this technique although great variability still existed. It was decided, therefore, to modify the acetylcholine method so as to assure the measurement of drug-induced activity and to quantify this activity on an intensity and duration basis.