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.

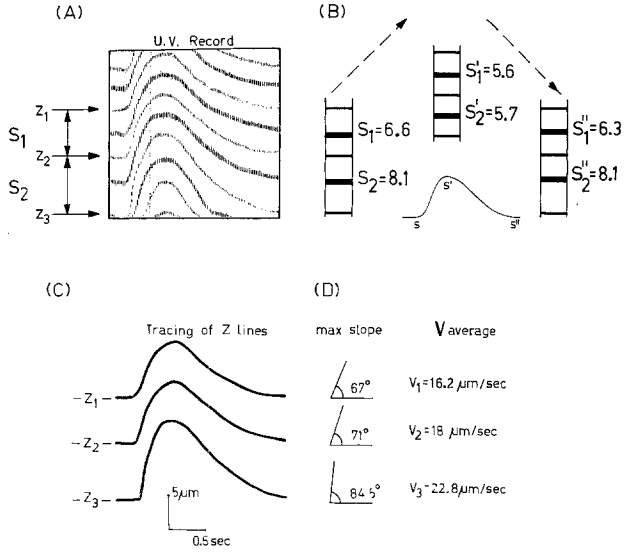

Fig. 2. A) Actual UV record of single contraction of a portion of muscle fibre in response to single shock stimulation obtained with the system described in Figure 1. Z, Z-lines of isolated living single muscle fibre; S_1, sarcomere 1; S_2, sarcomere 2. B) Diagramatic representation of the portion of 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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[9] H. E. Huxley, *The Structure and Function of Muscle* (Ed. G. H. Bourne; Academic Press, New York 1972), vol. 1, p. 331.

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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.

Table I. Reproducibility of 7 consecutive periods of atrial fibrillation produced at 15 min intervals (11 experiments)

Mean duration of atrial fibrillation (± S.E.)	7′32″[a] ± 23″	7′40″ ± 18″	7′04″ ± 15″	6′44″ ± 18″	7′24″ ± 30″	6′59″ ± 15″	8′00″ ± 21″

[a] The symbol ′ refers to min and the symbol ″ refers to sec.

Materials and methods. Mongrel dogs of both sexes weighing between 7 and 9 kg were used in these experiments. Anesthesia was induced with thiopental sodium, 20 mg/kg i.v., and maintained with a-chloralose, 60 mg/kg i.v. The animals were intubated with a cuffed endotracheal tube and respiration was maintained by a Harvard positive-pressure respiratory pump. A femoral artery and vein were catheterized for recording of mean arterial pressure and for i.v. injection, respectively. The electrocardiogram was monitored via Limb Lead II.

A right-sided thoracotomy was performed at the 4th interspace and the pericardium incised over the right atrium. The incised pericardium was then sutured to the thoracic wall to expose the atrium and to prevent contact from interfering with the results of the experiment.

Atrial flutter or fibrillation was induced by placing 2 drops of a 10% aqueous solution of acetylcholine directly on the right atrium and then stroking the area gently with a blunt spatula. The period of atrial fibrillation was determined by noting the return to normal sinus rhythm on the electrocardiogram. An interval of 15 min was allowed between the reversion to normal sinus rhythm and the induction of the next arrhythmia. 2 control periods of fibrillation or flutter were obtained. When the third period of tachyarrhythmia was induced, it was allowed to proceed for 10 sec to insure its onset and then the test compound was injected i.v. If a significant decrease in fibrillation time occurred, the duration of the activity was determined by inducing the tachyarrhythmia at 15-min intervals until the period of fibrillation returned to control values.

Quinidine sulfate, the compound evaluated in this study, was administered i.v. in cumulative doses calculated as the free base.

An analysis of variance of the data was undertaken using randomized block design[3].

Results. Although no attempt was made to prevent the acetylcholine solution from mixing with the pericardial fluid, a sufficient amount of the solution remained in contact with the atrium to allow the induction of atrial fibrillation. In control experiments, atrial fibrillation could not be induced solely by stroking the atrium.

The atrial fibrillation induced by the topical application of acetylcholine and stroking results in an irregular R-R interval of the electrocardiogram, and a decrease in femoral arterial pressure. The periods of atrial tachyarrhythmia induced by acetylcholine and stroking are reproducible as can be observed in Table I. These data summarize the results obtained with 7 consecutive periods of atrial fibrillation in 11 anesthetized dogs. An analysis of variance indicated that there was excellent reproducibility of the duration of the atrial arrhythmia within any one dog ($p > 0.1$). However, there was a very significant variation between dogs ($p < 0.005$). Because of this good reproducibility within animals, each dog can be used as its own control.

Quinidine sulfate was evaluated for potency and duration by this method in 6 animals and the results are summarized in Table II. As these results indicate, a slight decrease in the period of fibrillation was observed at a dose of 5 mg/kg i.v. At the 10 mg/kg i.v. dose, very pronounced activity was observed in each of six experiments. In these experiments, the period of fibrillation appeared to return to control within 15 min. The period of tachyarrhythmia after the 10 mg/kg i.v. dose increased in duration with consecutive inductions but plateaued at a time slightly lower than the control value. This happens occasionally but is interpreted as a return to control.

Discussion. Evidence indicates that the atrial tachyarrhythmia induced by acetylcholine is the result of a hyperpolarization and a conduction block[4]. This block would be localized in the area of direct contact with the acetylcholine. The tachyarrhythmia could then be caused by re-entry waves which are facilitated by delayed conduction[5].

This type of arrhythmia should be particularly sensitive to agents such as quinidine, which decrease intra-atrial conduction velocity and increase the effective refractory period. The former quality would decrease the number of impulses transmitted around a conduction block and the increase on effective refractory period would cause the head of the impulse to extinguish itself in its tail[6].

The technique, itself, allows the induction of atrial fibrillation or flutter at 15-min intervals, with the periods of arrhythmia being readily reproducible. This modification should allow one to compare both the potency and duration of potential of anti-arrhythmic agents.

Table II. Effect of quinidine sulfate on the duration of atrial fibrillation in 6 experiments

Quinidine sulfate (mg/kg i.v.)	Immediately after dose mean recovery period (+ S.E.)
0	Control I 8′15″ ± 17″
	Control II 8′45″ ± 54″
2.5	7′24″ ± 29″
5.0	6′28″ ± 38″
10.0	24″ ± 12″
	5′58″ ± 50″ 6′38″ ± 5″

Résumé. La méthode d'induction de fibrillation auriculaire par l'acétylcholine est couramment utilisée pour tester l'action de substances anti-arythmiques. Nous avons modifié cette méthode de telle sorte que la fibrillation peut être induite à plusieurs reprises, à 15 minutes d'intervalle, chez le chien anesthésié. Ceci permet la détermination de l'efficacité et de la durée d'action dans des conditions bien contrôlées.

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[1] D. SCHERF and F. B. CHICK, Circulation *3*, 764 (1951).
[2] W. J. SCHALLEK, J. Pharmac. exp. Ther. *105*, 291 (1952).
[3] R. G. D. STEEL and J. H. TORRIE, *Principles and Procedures of Statistics* (McGraw Hill, New York 1960), p. 132.
[4] W. TRAUTWEIN, Pharmac. Rev. *15*, 277 (1963).
[5] G. K. MOE and J. A. ABILDSKOV, Am. Heart J. *58*, 59 (1959).
[6] Y. WATANABE and L. S. DREIFUS, J. Pharmac. exp. Ther. *159*, 146 (1968).