not more than 6 h. For determination of catalatic activity FEINSTEIN's[9] perborate method with slight modifications was used.

The screening procedure was carried out by adding 0.003 ml blood to 5 ml 2.5% perborate solution. Those cases, unable to decompose this amount of perborate within 10 min, were further evaluated by using decreasing amounts (4, 3, 2, and 1 ml) of the same solution. This procedure permits the detection of activity levels corresponding to intervals of 0–20; 20–40; 40–60; and 60–80% of the normal level. No better accuracy was attempted for the purpose of this study. A group of 20 normal subjects was studied by this method. Their average catalase activity was 0.526 ± 0.018 ($\overline{X} \pm 5$) expressed as mM of decomposed perborate.

Results. Among the 10,009 blood samples of different individuals, 26 were found to have an enzymatic activity below the normal range. They were distributed as follows: 20–40% interval 4 samples; 40–60% interval 20 samples and in the 60–80 interval 2 samples. The percentage of hypocatalasemia is therefore 0.26%, which means one per 384 individuals. If we take all the cases together, not considering the posibility of the existence of several alleles, the frequency would be 0.0013, with a confidence limit of 0.008 to 0.0019. We have not found cases of acatalasemia (term coined by TAKAHARA and MIYAMOTO[7]), and only one of the individuals was affected with pyorrhea, a woman whose catalatic activity turned out to be lowest in this screening. A daughter of this woman was also affected with the same illness, but her catalatic activity was normal.

Discussion. The data available do not allow one to decide to which type of AEBI's[5] classification the hypocatalasemia cases found in Spain may be attributed. The variability of catalase activity found in this population may be of genetic origin, such as the presence of several alleles, or it may be due to experimental variation since the number of cells employed for this analysis was not exactly determined. In cases of ferropenic and haemolytic anaemia, normal values are found when catalatic activity is related to haemoglobin content (PAUL and ENGSTED[10]). It is for this reason that, when measuring absolute values, these subjects may appear as hypocatalatic. It was assumed that the haemoglobin concentration would be approximately normal in all the samples analyzed.

It may also be that hypocatalatic subjects have been overlooked. If there is an overlap between the activities of hypocatalatics and normals, those individuals with an activity of over 80% may have been scored as normals. Due to this overlapping, it is possible that our figures underestimate the real number of hypocatalatics (i.e. in cases of group IIIb deficiency, AEBI[5]). On the other hand, the presence of enzyme inhibitors in blood serum, as demonstrated by PAUL and ENGSTED[10], may contribute to an overstimation.

The figure for the frequency of the acatalasemia gene (0.0013) probably represents a maximal value. Nevertheless our values are in agreement with the assumption that – although the average gene frequency seems to vary from country to country (TAKAHARA 1967 in AEBI[5]), the acatalasemia gene is of worldwide distribution.

Resumen. Se ha estudiado la actividad catalásica en la sangre de 10.009 españoles residentes en Madrid, por el método de FEINSTEIN[9]. Se encontraron 26 individuos cuya actividad catalásica oscila entre el 20% y el 80% de la normal. Estos individuos se consideraron como hipocatalasémicos. Se comparan estos datos con los de otros autores y se discute su posible significado.

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Difference in Mechanical Properties of Adjacent Sarcomeres in Single Striated Muscle Fibres of the Horseshoe Crab (*Tachypleus gigas*)

Striated muscle fibres contain both contractile and visco-elastic elements. Such visco-elastic elements could be made to reveal themselves when the muscle is overloaded i.e. under so-called isometric conditions. HILL[1] suggested that during isometric contraction the actively contractile elements would shorten at the expense of the passive elastic elements. Since then, several methods have been designed to test his suggestion[2–6]. However all the methods used to measure the properties of the elastic elements were based on estimations from the force-velocity relation and the relation between tension and time derivative of isometric tension during tetanus[7]. In this paper we have used the method of simultaneous measurements of adjacent sarcomere length changes to study the behaviours of adjacent sarcomeres in single striated muscle fibres during isometric twitch[8a]. The observed difference in the average velocities of adjacent Z lines in some special cases is considered to be an index of the difference in the visco-elastic elements of adjacent sarcomeres.

Materials and methods. The experiments were performed on the short accessory muscle (SAM), a receptor muscle[8b], in the walking leg of the Asiatic horseshoe crab, *Tachypleus gigas*. These muscle fibres were selected for study because of their broad striations (2–12 μm) and small diameters

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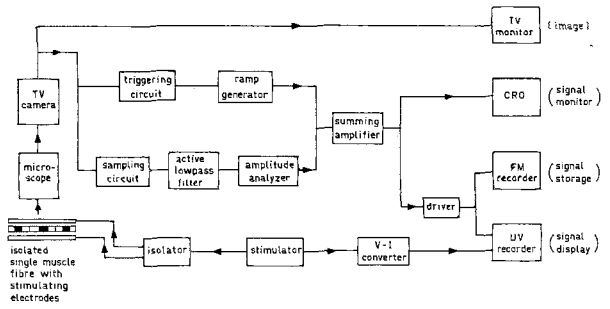

Fig. 1. Block diagram for measurements of sarcomere lengths and velocities of adjacent Z lines during isometric twitch of isolated striated single muscle fibre.

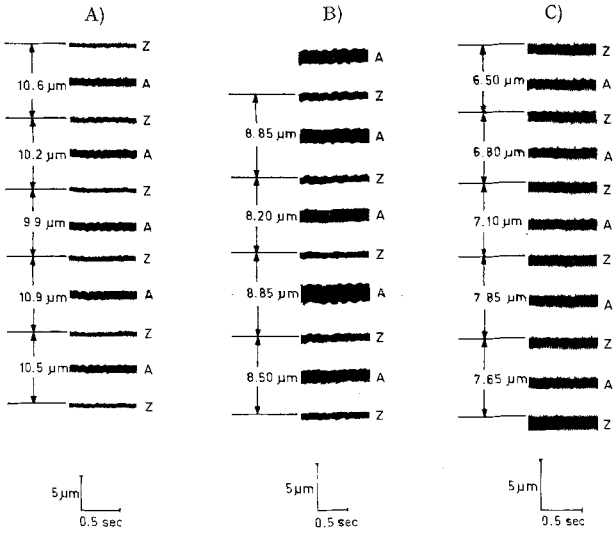

Fig. 2 A–C. UV records of adjacent sarcomere lengths from 3 different muscle fibres in the resting state. In A) and B), A bands are indicated by broad stripes and Z lines by thin stripes. Sarcomere lengths are measured from Z–Z intervals, and x-axis is the time axis. Ripples indicate very slight spontaneous movements. In C), the signals have been enhanced by an amplitude analyzer.

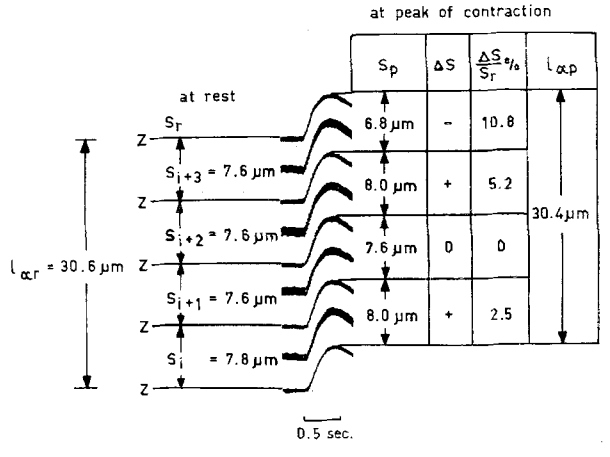

Fig. 3. UV record of 4 sarcomeres bounded by 5 Z lines during isometric twitch of a single muscle fibre. The lengths of the respective sarcomeres are measured at rest, and at the peak of contraction. The segment l_α under observation as a whole shortens very little, only from 30.6 µm to 30.4 µm. However, variations in the individual sarcomere length at peak of contraction are considerable. Thus S_{i+3} is only 6.8 µm, while S_i and S_{i+2}, each measures 8.0 µm. l_α = total length of the 4 sarcomeres; S_r = individual sarcomere length at rest; S_p = individual sarcomere length at peak; $\Delta S = S_p - S_r$; (+) = lengthening; (0) = no change; (−) = shortening.

(2–16 µm)[9]. Isolation of single muscle fibres was performed under a binocular dissecting microscope (Carl Zeiss Model III). The isolated single muscle fibre was then placed in a special lucite chamber containing oxygenated sea water at pH 7.2–7.4 and specific gravity of 1018–1023. Sometimes horseshoe crab Ringer made according to Wyse's formula, containing 25.9 g/l NaCl; 1.0 g/l KCl; 3.64 g/l $CaCl_2 \cdot 2H_2O$; 3.06 g/l $MgSO_4 \cdot 7H_2O$, and buffered by 0.5 *M* solutions of NaOH at 0.96 ml/l and H_3BO_3 at 17.6 ml/l was used[10]. The origin of the fibre was fixed by a stainless steel spring clip and the tendinous insertion was attached by a stainless steel micro-hook to a micromanipulator constructed in our laboratory. The isolated muscle fibre could then be stretched to varying degrees of the resting length (L_o). Most of the muscle fibres studied were stretched to about 120% of the resting length. The fibre was then transilluminated and observed under a phase-contrast microscope (Olympus, Vanox NH 40×/0.65 or Leitz UMK 50/0.60 objective) connected to a closed-circuit TV system (ITC Model CTC-5000). Adjacent sarcomeres along any region of the single muscle fibre were then scanned by the vidicon and transformed into video signals which varied with the optical densities of the fibre. Processing of the video signals was essentially similar to that reported by HWANG et al.[9] except that a sampling circuit and an amplitude analyzer were introduced into the circuit (Figure 1). The sampling circuit was designed so that any vertical strip of the image appearing on the TV monitor, i.e. a fraction or the whole width of the muscle fibre, could be sampled and the video signals singled out for processing. An amplitude analyzer (Frederick Haer & Co. Model 40-75-5) was used to enhance the signals from the Z lines whenever necessary. Comparison of the UV recordings with the actual photomicrographs at high magnification indicates that, provided the sarcomeres are longer than 4 µm, the sarcomere lengths as measured by our technique are accurate to 0.1 µm. Sarcomere lengths or their changes, as indicated by the intervals between adjacent Z lines were then displayed on a UV recorder (SE Laboratory Model 2006). The y-axis of the UV record would indicate displacement of Z lines or changes in sarcomere lengths; and the x-asix would be the time axis. Supramaximal stimulus was delivered through a pair of fine bright platinum electrodes insulated except portions of the tips, placed one on either side of the fibre. Rectangular pulses were provided by an electronic stimulator (Grass Inst. S88) through an isolation circuit.

Results. In the short accessory muscle (SAM), the sarcomere lengths were found to differ from one fibre to the next and even within the same fibre. Figure 2A to C shows the UV records from 3 different isolated fibres at rest, with each fibre set at 120% of L_o. In Figure 3C the original video signals emitted from the Z lines were relatively weak and indistinct. An amplitude analyzer was used to enhance the signals. Closed-circuit TV scanning of over 50 different single muscle fibres showed that sarcomere lengths could vary from 2 µm to 15 µm (mean = 8.1 ± 3.4 µm S.D.). Within any one muscle fibre, the variation in sarcomere lengths was smaller. The standard deviations for sarcomere lengths within each muscle fibre ranged from ± 0.74 to 1.29 µm; and within any 60 µm along a single fibre, adjacent sarcomeres seldom differed by more than 10% at rest.

With single supramaximal shock, it was readily observed that under isometric conditions, some sarcomeres shortened, while others lengthened or remained constant

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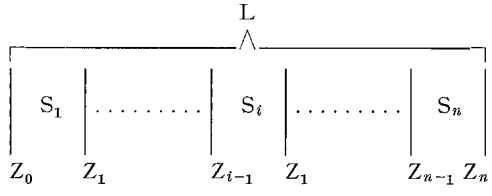
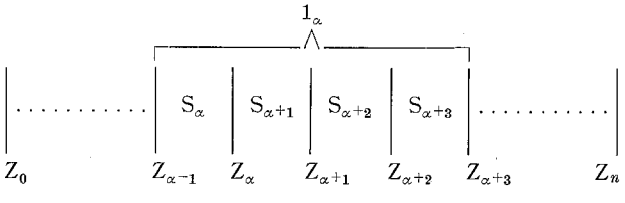
in length. A more precise description of the single muscle fibre during isometric twitch is formulated here below. Each muscle fibre is made up of a series of sarcomeres (approximately 800–1200). Each sarcomere S_i is bounded by two Z lines, Z_{i-1} and Z_i.



where: Z_0 = first Z line (origin), and Z_n = last Z line (insertion), L = total length of the muscle fibre.

During isometric twitch, with $\Delta L = 0$, we have observed that S_i could assume any value. Figure 3 shows one such example. The UV record shows 4 sarcomeres within a single muscle fibre, S_i, S_{i+1}, S_{i+2} and S_{i+3}. At the peak of contraction, both sarcomeres S_i and S_{i+2} elongated although to different extents. Sarcomere S_{i+3} shortened by 10.8% while sarcomere S_{i+1} remained unchanged. As the initial sarcomere lengths were not uniform even at rest, any attempt to analyze the contributions made by each sarcomere to the single muscle fibre system as a whole, be they active or passive, would be extremely complex. An attempt was therefore made to select sarcomeres whose lengths in the resting state were exactly identical within the limits of our measuring technique. We have therefore selected cases or segments of single muscle fibres whose sarcomeres were initially uniform in lengths for study. The segment 1_α selected for study must satisfy the following requirements.



(a) $1_\alpha = \alpha \sum_{i=\alpha}^{\alpha+3} S_i$

(b) $\Delta 1_\alpha = 0$

(c) $S_\alpha = S_{\alpha+1} = S_{\alpha+2} = S_{\alpha+3}$

(d) $\Delta S_\alpha = 0 = \Delta S_{\alpha+3}$

(e) $|\Delta S_{\alpha+1}| = |\Delta S_{\alpha+2}|$

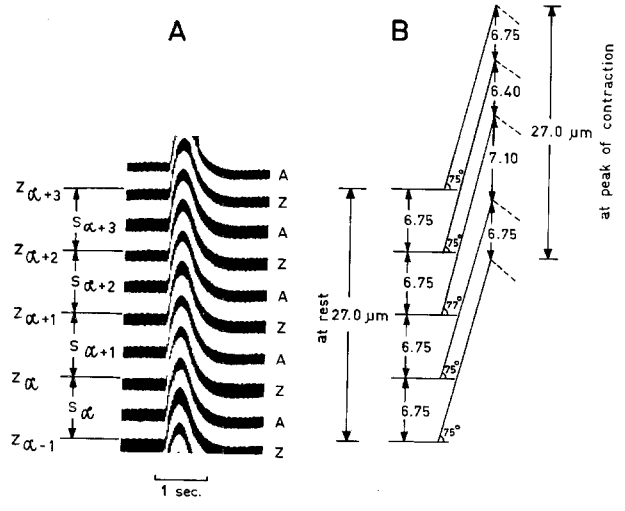


Figure 4 shows the actual UV recording of one such segment 1_α during the course of an isometric twitch of the single muscle fibre. Since 1_α was the same at rest and at the peak of contraction, the sum total of all the forces generated within the segment 1_α must be equal and opposite to the external forces applied at $Z_{\alpha-1}$ and $Z_{\alpha+3}$. As the initial lengths of the 4 sarcomeres were equal within the segment 1_α, and as $\Delta S_\alpha = 0 = \Delta S_{\alpha+3}$,

$$\left(\frac{dZ_{\alpha-1}}{dt}\right)_{av.} = \left(\frac{dZ_\alpha}{dt}\right)_{av.} = \left(\frac{dZ_{\alpha+2}}{dt}\right)_{av.} = \left(\frac{dZ_{\alpha+3}}{dt}\right)_{av.}$$

$$\left[\left(\frac{dZ_\alpha}{dt}\right)_{av.} - \left(\frac{dZ_{\alpha+1}}{dt}\right)_{av.}\right]$$

would be indicative of the difference in the visco-elastic elements resident in $S_{\alpha+1}$ and $S_{\alpha+2}$. Figure 4 shows that 1_α was 27.0 µm at rest and also at the peak of contraction, with each sarcomere measuring 6.75 µm initially. Sarcomere $S_{\alpha+2}$ shortened from 6.75 µm to 6.40 µm while sarcomere $S_{\alpha+1}$ lengthened from 6.75 µm to 7.10 µm. The difference in slope of 2° between $(dZ_{\alpha+1}/dt)_{av.}$ and the adjacent Z lines would indicate the difference in visco-elastic elements between the 2 adjacent sarcomeres.

Discussion. The heterogeneous behaviour of muscle when stimulated under isometric condition is well known[2,11]. These could be due to the difference in either the force generators or the visco-elastic elements in different parts of the muscle, or both. Markers placed on contracting muscles have shown that the muscle fibres do contribute to the series compliance[5]. In the intrafusal muscle fibres of mammalian muscle spindles, evidence of regional constitutional difference in the contractile apparatus has also ben presented[12,13]. The contractility and quality of the myofilaments and related intracellular structures could differ from one region to the next and such regional variations in the subcellular constituents could in turn affect the visco-elastic properties as well as the contractile properties[14,15]. By recording the velocities of adjacent Z lines in the course of an isometric twitch in cases in which the adjacent sarcomeres are of uniform lengths, we believe we have been able to demonstrate different visco-elastic elements inherent in different sarcomeres. Electron microscopic studies have shown that this muscle exhibits complicated subcellular constituents[16]. Dyads and triads coexist in this muscle and are present in variable number from one sarcomere to the next. Some myofilaments exhibit peculiar periodicities and the Z lines are variably adhered to the sarcolemma (Hwang and Wong, unpublished data). Thus the morphological substrate for the varied behaviour of adjacent sarcomere seems to exist. This study has therefore provided further evidence that striated muscle even at the level of single muscle fibre is still an extremely heterogeneous system. Even in the case where the initial

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Fig. 4. A) UV record of 4 sarcomeres during isometric twitch. In B), the average velocities of the respective Z lines are expressed in angles. Note that $Z_{\alpha+1}$ which lies between sarcomere $S_{\alpha+1}$ and sarcomere $S_{\alpha+2}$ moves at a verlocity different from the adjacent Z lines.

sarcomere lengths are identical, adjacent sarcomeres still could behave differently in response to single electrical shock. This could be attributed to a difference in the visco-elastic elements.

For the sake of convenience and for ease of analysis, muscles are studied either under isotonic condition or isometric condition. Under isotonic condition, velocity is usually expressed in units of L_o/sec. This at best merely represents average velocity of sarcomere shortening in units of sarcomere length/sec[17]. CLOSE[18] has estimated the speed of shortening per sarcomere by dividing the speed of shortening of the whole muscle by the average number of sarcomeres per muscle fibre within a muscle. His estimations, however, assumed that, a) all the muscle fibres were orientated in the same direction, b) all the muscle fibres were of uniform length, c) each muscle fibre had a uniform number of sarcomeres[19], d) every sarcomere length was uniform, e) the force-generator in each sarcomere was the same and f) the visco-elastic property of each sarcomere was the same.

Likewise in isometric measurements, even with single muscle fibres, GORDON et al.[20] in their studies on the length-tension curves must also assume the validity of c), d), e) and f). Our study indicates that at least in the striated muscle fibres in the horseshoe crab, b), c), d), e) and f) are by no means sound and valid assumptions all the time.

Zusammenfassung. An Beinmuskulatur des Pfeilschwanzkrebses *Tachypleus gigas* wurden während isometrischen Einzelzuckungen die Distanzen zwischen benachbarten Z-Scheiben und A-Banden unter Verwendung einer Televisionsanordnung ausgemessen. In einer ruhenden Einzelfaser erwiesen sich die Längen der einzelnen Sarkomeren als nicht einheitlich. Während einer isometrischen Einzelzuckung verkürzten sich gewisse Sarkomere, während andere in die Länge gezogen wurden. Bei gleicher Ruhelänge von benachbarten Sarkomeren war die Geschwindigkeit der Verkürzung in der Regel ungleich. Dies wird auf Unterschiede im visco-elastischen Verhalten zurückgeführt. Die Resultate zeigen deutlich, dass selbst aus dem Verhalten von Einzelfasern nicht ohne weiteres auf das Verhalten von einzelnen Sarkomeren geschlossen werden darf.

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Effects of Temperature on Osmotic Responses and on Transmembrane Efflux of Urea and Sodium in Vascular Smooth Muscle Cells

In previous studies of osmotic responses in smooth muscle of rat portal vein, we have described an intimate relation between the changes in cell volume and the spontaneous electrical and mechanical activity[1-3]. Anisosmolar solutions containing urea caused characteristic transient changes in activity. A close correlation was demonstrated between the time course of these reponses and the rate of penetration of the molecule through the cell membranes as studied directly by ^{14}C-urea[2]. The present report is concerned with the effects of temperature on such contractile responses and on membrane permeability.

Figure 1 illustrates increases of contractile activity observed in rat portal vein on return to standard solution

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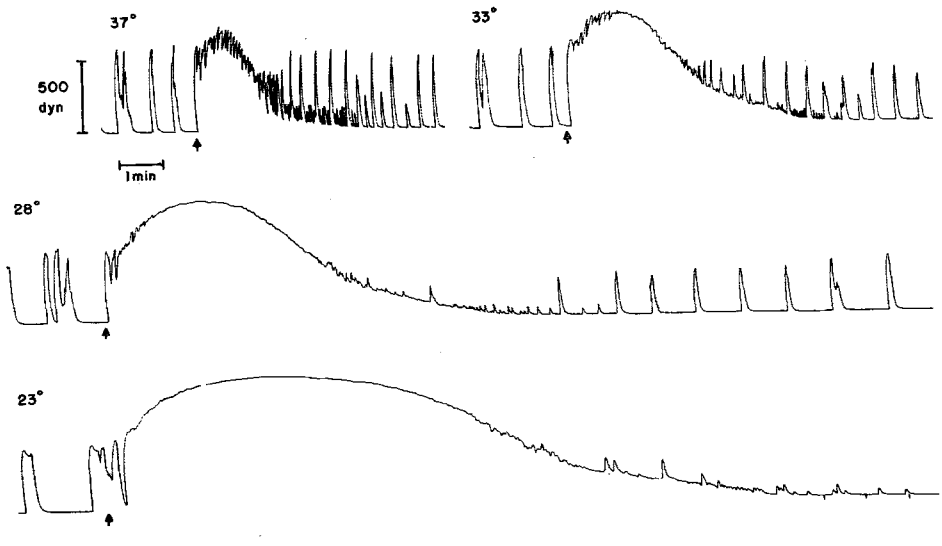

Fig. 1. Time course of the transient increase of contractile activity observed in a portal vein preparation on return to standard Krebs solution (arrows), after previous equilibration in a hyperosmotic solution with 100 m*M* urea. The duration of the excitatory response is markedly increased with decreasing temperature.